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ARTICLE

Endophallus inflation: a potential taxonomic tool for the subfamily Scolytinae (Coleoptera: Curculionidae)

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Abstract

The taxonomy of the subfamily Scolytinae has traditionally been based on external morphological attributes corresponding to the elytral declivity, head, and pronotum. Some traits from the general morphology of the aedeagus and spermatheca have been proposed in scarce genera. In this study, we explore and improve a technique of endophallus inflation to apply it in Scolytinae members and to describe its morphology for the first time in 16 species from *Dendroctonus* Erichson, *Ips* DeGeer, and *Phloeosinus* Chapuis. These taxa display differences in the attachment types of endophallus and two distinct inflation and retraction mechanisms. Our results support the use of the internal sac as a taxonomic feature in Scolytinae because each tribe, genus, and species display a particular morphological pattern. The results also indicate use of the internal sac for *Dendroctonus* taxonomy supports phylogenetic inferences.

Introduction

The taxonomy of the subfamily Scolytinae has traditionally been based on external morphological attributes that correspond to the elytral declivity, head, and pronotum because their variation allows species identification and because these body parts display cuticle elements easily observable and quantifiable in terms of abundance, size, and distribution (Hopkins 1909; Wood 1982; Hulcr *et al.* 2015).

In insects, particularly in Coleoptera, male genital attributes are important for taxonomy (Coca-Abia and Robbins 2006; Pardo-Diaz *et al.* 2019; Yang *et al.* 2020; Gao and Coca-Abia 2021); however, these attributes have been poorly integrated in bark beetle studies due to the difficulty of manipulating small specimens and structures (Hulcr *et al.* 2015). Although the genitalia have been explored in Scolytinae for taxonomic purposes (Wood 1957; Hopping 1963; Lanier 1972, 1987; Pajares and Lanier 1990; Lanier *et al.* 1991; Furniss 1996; Mandelshtam *et al.* 2006, 2012), detailed and focused study of the morphology of the endophallus has not been carried out to date. In a few taxa, specialised parts of male genitalia can provide good taxonomic traits. These parts include the

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aedeagus, seminal rod, and anchor shape in *Camptocerus* Dejean (Smith and Cognato 2010), *Carphobious* Blackman (Cognato and Smith 2023), members of the Cryphalini (Johnson *et al.* 2020; Justesen *et al.* 2023), *Dendroctonus* Erichson (Rios-Reyes *et al.* 2008; Armendáriz-Toledano *et al.* 2014; García-Román *et al.* 2019, 2022; Valerio-Mendoza *et al.* 2019), the Hylastini (Mandelshtam and Petrov 2019), the Hypoborini (Jordal 2021b), the Micradicini (Jordal 2021a), and *Xyloctonus* Erichhoff (Jordal 2024), and the receptacle in *Xyleborus* Eichhoff (Pérez-Silva *et al.* 2015), whereas in other beetles, such as *Scolytus* Geoffroy, male genitalia display extremely conserved patterns (Johnson *et al.* 2008; Smith and Cognato 2014).

A little-explored structure with taxonomic potential is the endophallus, an eversible membranous sac located within the aedeagus (Tuxen 1970; Nichols 1989). During the copulation process, the endophallus is placed inside the bursa copulatrix, functioning as a lock-and-key system and as a prezygotic isolation mechanism (Düngelhoef and Schmitt 2010), making it useful for defining species boundaries and estimating phylogenetic relationships (Coca-Abia 2007; Düngelhoef and Schmitt 2010; Sasabe *et al.* 2010; Erbey and Candan 2018). In several Coleoptera taxa, the endophallus is a powerful taxonomic tool for delimiting and identifying problematic species (Danilevsky and Kasatkin 2004; Bollino and Sandel 2017).

The first comparative study of the anatomy of the male genitalia in the order Coleoptera corresponds to Sharp and Muir (1912). They described characteristics of the shape and structures of the endophallus, incorporated a technique for its eversion, and emphasised the importance of including it in taxonomic studies. Since this work was published, the endophallus morphology has contributed significantly to the understanding of the taxonomy of different beetle families, such as Lucanidae (Imura 2007), Glaphyridae (Uliana and Sabatinelli 2010; Bollino and Ruzzante 2015), Chrysomelidae (Bukejs and Anichtchenko 2019; Daccordi *et al.* 2020), Carabidae (Anichtchenko 2010; Janovska *et al.* 2013), Curculionidae (Bollino and Sandel 2017; Meregalli *et al.* 2020), and Cerambycidae (Danilevsky and Kasatkin 2004; Yamasako and Ohbayashi 2011), among others.

In some taxa of Scarabaeoidea, the everted and noninflated endophallus's structural characteristics, such as sclerites, spines, and silks, have been used as attributes for phylogenetic inferences (Coca-Abia 2007). In Scolytinae, the endophallus has been studied only in *Dendroctonus monticolae* Hopkins (= *D. ponderosae*) (Cerezke 1964): that study focused on describing the inflated endophallus and the musculature associated with its eversion movement but did not present the technique used for inflation.

The diversity of the Coleoptera groups in which the endophallus has been studied allowed the development of several inflation techniques using different substances to inflate and fill this structure. The Berti–Vachon technique, or "air filling" (Bontems 2013), is one of the most commonly used because it recovers the endophallus shape and maintains the integrity of the structures of the internal sac; however, its execution is complicated, especially in small specimens. Another commonly used technique is that of Berlov (1992), which consists of filling the sac with toothpaste. Although this method recovers the endophallus shape, details of the sclerotised structures are lost because of the toothpaste's colour and the internal sac loses volume during the drying process. To avoid this problem, Uliana and Sabatinelli (2010) proposed a modification to Berlov's technique that adds micronised silica to reduce these effects. Yamasako and Ohbayashi (2011) showed that using glycerin as a filling substance is also effective; the only disadvantage is that the structure cannot be preserved dry as it can with the previous techniques. Van Dam (2014) developed a new inflation technique for small curculionids, applicable to both fresh and museum specimens; this method consists of cleaning the aedeagus with an enzymatic solution and then using K-Y gel to fill the endophallus. Although Van Dam's (2014) technique yields effective results and maintains the transparency of the membrane, the sample also cannot be kept dry.

In this study, we propose modifications to Van Dam's (2014) technique for the study of the endophallus of Scolytinae members. In addition, we describe the anatomy and morphology of the internal sac for the first time in 16 species from *Dendroctonus* Erichson, *Ips* DeGeer, and *Phloeosinus* Chapuis, emphasising the sac's usefulness in taxonomy. We also describe and discuss the inflation patterns of the internal sac.

Materials and methods

Sixty specimens corresponding to 16 species of three genera of Scolytinae were analysed. Of these, 10 were *Dendroctonus* Erichson spp.: *D. adjunctus* Blandford, *D. approximatus* Dietz, *D. barberi* Hopkins, *D. frontalis* Zimmerman, *D. mesoamericanus* Armedáriz-Toledano and Sullivan, *D. parallelocollis* Chapuis, *D. pseudotsugae barragani* Furniss, *D. vitei* Wood, *D. rhizophagus* Thomas and Bright, and *D. valens* (LeConte). Four were *Phloeosinus* Chapuis spp. (Coleoptera: Curculionidae): *P. baumanni* Hopkins, *P. deleoni* Blackman, *P. tacubayae* Hopkins, and *P. serratus* LeConte. Two were *Ips* DeGeer spp. (Coleoptera: Curculionidae): *I. lecontei* Swaine and *I. calligraphus* (Germar) (Table 1). The specimens examined included those obtained from infested trees in 21 localities in Mexico and Honduras, as well as museum specimens, some of which were mounted and some of which were preserved in alcohol. Museum specimens were borrowed from the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico, and the collected specimens were deposited in it. Specimens from different populations were included in *D. valens* and *P. serratus* because these species are known for their cryptic diversity (Ramírez-Reyes *et al.* 2023).

To sex and identify specimens, we used dichotomous keys. *Dendroctonus* spp. were sexed based on external body traits and the shape of the seventh tergite (Armendáriz-Toledano *et al.* 2015) and by the presence of stridulatory apparatus in males (Lyon 1958). Species identification was made based on external and internal morphology attributes, such as the presence or absence of frontal tubercules, the length and abundance of pubescence of elytral declivity (in *D. adjunctus* and *D. approximatus*; García-Román *et al.* 2019), the presence of coarse rugosities on the elytral interspaces and impressed striae, and the short pubescence on the elytral declivity (in *D. barberi*; Valerio-Mendoza *et al.* 2019), the shape of the lateral margins of the epistomal process and pronotum (in *D. parallelocollis* and *D. pseudotsugae barragani*; Armendáriz-Toledano and Zúñiga 2017; García-Román *et al.* 2019), characteristics of the antennal club (in *D. rhizophagus, D. valens*, and *D. vitei*; Armendáriz-Toledano *et al.* 2014), and characteristics of the male genitalia such as the seminal rod and anchor (in *D. frontalis* and *D. mesoamericanus*; Armendáriz-Toledano *et al.* 2014, 2015).

Phloeosinus specimens were sexed based on whether the eighth tergite was visible (males) or not (females; Hopkins 1905; Cervantes-Espinoza *et al.* 2023). The species were identified based on external morphological characters (Blackman 1942; Wood 1982). Characters from the head were used, including frons shape, the elevation of the carina concerning the epistomal margin, and the shape of antennal sutures, the density of pubescence and punctuations of pronotum, the shape of elytral declivity, the width of interstriae, and the number and shape of the declivital teeth (in *P. baumanni, P. deleoni, P. serratus*, and *P. tacubayae*)

Finally, the *Ips* specimens were identified using the dichotomous keys of Lanier (1987), Douglas *et al.* (2019), and LaBonte and Valley (2019). The *I. calligraphus* specimens were sexed based on the hook shape of the third spine of the elytral declivity, whereas *I. lecontei* males were identified based on the presence of bifurcate tubercles at the centre of the epistomal margin.

Male adults were dissected to obtain the genitalia. The methods used to obtain the aedeagus differed according to how the specimens were preserved (dry, alcohol, or fresh) and are described below.

Rehydration of dry-mounted specimens. For optimal extraction of the genitalia, the specimens were first rehydrated using a solution of distilled water and 50% commercial meat tenderiser to soften them. The ingredients of the meat tenderiser, as indicated on the product label,

Table 1. Locations, geographical coordinates, hosts of examined *Dendroctonus*, *Ips*, and *Phloeosinus* specimens, and the collection where they are found. Specimens supporting these records were deposited in the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México

Species	Country	Locality	Latitude	Longitude	Host
D. adjunctus	Mexico	Nevado de Colima	19.568835	–103.6165512	*Pinus hartwegii*
D. approximatus	Mexico	Oaxaca, Portillo, Cajones (OPC)	16.2389472	–95.4774778	*Pinus* sp.
D. barberi	Mexico	Nuevo León, Galeana-Carretera Linares-Galeana la 'Y'	24.7791667	–99.955	*Pinus engelmannii*
D. frontalis	Mexico	Michoacán, Charo, La mesa	19.6	–100.93	*Pinus* sp.
D. mesoamericanus	Honduras	Francisco Morazán	14.0161111	–86.8955556	*Pinus ocarpa*
	Mexico	Michoacán, Villa Madero, Balcones	19.0833333	–100.942222	*Pinus pringlei*
D. parallelocollis	Honduras	Potrerillos, Zihuatepeque (HPZI)	14.5869892	–86.1699111	Lindgren trap
D. pseudotsugae barragani	México	Arteaga, Coahuila	25.4372222	–99.2916667	*Pseudotsugae menziessi*
D. valens	Honduras	Comayagua, Villa de San Antonio; Chagüite Grande	14.5969444	–86.1405556	Lindgren trap
	Mexico	Ajusco, Parque Ejidal San Nicolas Totolapan.	19.2525	–98.7566667	*Pinus* sp.
D. vitei	Mexico	Nuevo León, Iturbide, Los Pinos del Sur	24.7166667	–98.1	*Pinus teocote*
D. rhizophagus	Mexico	México, Durango, El Salto,	23.763	–105.365	*Pinus duranguensis*
P. deleoni	Mexico	Oaxaca, San Juan Bautista, Coixtlahuaca	17.65	–97.276861	*Pinus flaccida*
P. baumanni	Mexico	CDMX, Ciudad Universitaria.	–19.315	–99.181	*Hesperocyparis lusitanica*
P. tacubayae	Mexico	Iturbide, Ejido la Purísima	24.5422222	–98.175	*Hesperocyparis lusitanica*
P. serratus	Mexico	Nuevo León, Galeana	24.49	–100.07	*Hesperocyparis arizonica*
	Mexico	Nuevo León, Aramberri	24.8596667	–100.360333	*Juniperus saltillensis*
	Mexico	Nuevo León, Aramberri	24.8223611	–100.084333	*Juniperus coahuilensis*
I. lecontei	Mexico	Durango, La Victoria, Chavarría	–	–	*Pinus* sp.
I. calligraphus	Mexico	Nuevo León, Galeana, Camino a la Y de Abajo	24.7791667	–99.955	*Pinus* sp.

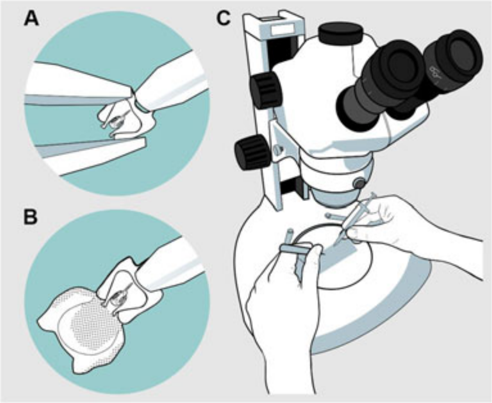

Figure 1. Endophallus inflation process: **A**, first, the forceps are fixed around the base of the basal orifice with the blunt-tipped syringe in the centre; **B**, then the endophallus is inflated; and **C**, the process is realised with a slide and syringe under stereoscopic microscope.

were iodised salt, dextrose, papain, and calcium stearate. The procedure consisted of placing the insects in vials with this solution and incubating them for 45–60 minutes at 60 °C.

Obtaining and cleaning the aedeagus. After rehydration, the dissection to obtain the aedeagus was performed for all specimens (preserved dry, preserved in alcohol, and fresh). The abdomen was removed and placed in a vial with a pancreatin solution to facilitate tissue digestion (Álvarez-Padilla and Hormiga 2007; Van Dam 2014); the samples were incubated for 1–2 hours at 37 °C, although they can also be left at room temperature for 2–3 days. Once the samples were clean, they were rinsed with distilled water and then preserved in alcohol. If the tissue did not dissolve completely, the samples were incubated for 30 minutes at 60 °C with 10% NaOH to finish cleaning and softening, then they were neutralised with 10% HCl, rinsed with distilled water, and preserved in 70% alcohol.

Endophallus inflation. Once the aedeaguses were clean, the endophalluses were inflated. Van Dam's (2014) technique was used, with modifications. An insulin syringe was used to inflate each endophallus: the tip of the needle was filed to a right angle, and the contour was filed to obtain a conical shape and to facilitate inflation. A lubricant K-Y gel was used as an inflation substance. Because of the gel's density, it was diluted to 50% dilution with 96% alcohol before inflation.

A drop of the gel solution was placed on a slide, and an aedeagus was placed in it. The procedure was performed using a stereo microscope. With the help of fine-tipped forceps, the body of the aedeagus was held, taking care not to crush it, and it was placed in a dorsal position or in a position that allowed easy manipulation for inflation. With the syringe, the gel was injected through the basal orifice of the aedeagus located between the apodemes (Fig. 1). Once the endophallus was everted, it was photographed from various aspects – dorsal, lateral, and ventral – for subsequent description. A compound microscope with a camera at 40×, 100×, and 400× magnification, depending on species size, was used to photograph the endophallus. Finally, the samples were preserved in a vial with 70% alcohol. For some species, the inflation process was video-recorded to capture the movement of the structures involved; however, in the case of the genus *Ips*, this was the only option to describe it due to the intrinsic characteristics of the endophallus (see Results). From the photographs and videos, drawings of the different views of the endophallus of all species were made.

The endophallus anatomy was described using the nomenclature of Cerezke (1964), Tuxen (1970), Nichols (1989), Yamasako and Ohbayashi (2011), Daccordi *et al.* (2020), and Meregalli *et al.* (2020). The definitions for describing the structures of the aedeagus and endophallus were based on the glossary of Cerezke (1964), Tuxen (1970), Nichols (1989), and

Armendáriz-Toledano *et al.* (2015). Use of the terms "aedeagus" and "penis" is restricted in the present study to the body part commonly known as the median lobe, which contains the accessory apparatus and the internal sac, and does not include the spicule and tegmen. A list of abbreviations of aedeagus terminology to describe endophallus morphology is presented in Supplementary material, file S1.

Results

Inflation

Of the 16 species analysed, two were only partially inflated because they were dry-preserved specimens with very small aedeaguses. In the *Dendroctonus* and *Phloeosinus* specimens, the inflated endophallus was maintained for days after the procedure, which facilitated their observation and description. In the *Ips* specimens, the endophallus maintained its shape only when the pressure of the injection fluid was constant: when the fluid was no longer applied, the sac retracted into the aedeagus, suggesting a stronger retraction mechanism (Supplementary material, video S1).

Morphology

Our results show that, in *Dendroctonus*, *Phloeosinus*, and *Ips*, the genital organ consists of three sclerotised structures – the spicule, tegmen, and penis (*sensu* Cerezke 1964) or aedeagus (*sensu* Nichols 1989). The spicule and tegmen lie adjacent to the penis but are attached ventrally to it by muscle and membranes. The tegmen is a small transversal segment, irregular in shape, that is attached to apodemes; the structure provides musculature support during eversion (Cerezke 1964). In *Dendroctonus*, the tegmen is a U-shaped structure located in the ventral region of the aedeagus close to the apodemes, whereas in *Phloeosinus*, it is V-shaped, and in *Ips*, it is a ring surrounding the aedeagus. The spicule is a small, parallel, needle-like spine attached by membranes to the outside of the aedeagus (Tuxen 1970); distally, the spine is bifurcated. Partially surrounding the aedeagus, the spicule supports the aedeagus. The spicule is similar in shape among all species, differing between the species primarily in size.

In the *Dendroctonus* and *Phloeosinus* spp., we found that the endophallus and an accessory apparatus are present within the aedeagus, the accessory apparatus consisting of the seminal rod and an anchor.

The aedeagus is a capsule with two orifices, the basal one in the proximal region and the apical one, or ostium, in the distal region. In the basal region are the apodemes, which correspond to a pair of thin extensions that support the spermatic duct; the length of apodemes varies among genera and species, mainly among *Ips*. The seminal rod and anchor conform to the accessory apparatus in *Dendroctonus* and *Phloeosinus*: these structures vary within species, mainly among *Dendroctonus*. Both structures are involved in the endophallus eversion process during copulation.

The endophallus in the taxa examined in the present study is a sac that is continuously connected to the distal end of the aedeagus. When the endophallus is fully everted and inflated, it looks like a semitransparent membrane that is composed of lobes (Fig. 2). During the eversion process, the endophallus exits through the ostium in *Dendroctonus,* driven by the movement of the accessory apparatus to which it is attached (Fig. 2A). In *Phloeosinus*, the endophallus ascends through the dorsal–distal region of the aedeagus, where it joins at the lateral folds, and in the dorsal–proximal region, it joins with the accessory apparatus (Fig. 2B). In both *Dendroctonus* and *Phloeosinus*, when the endophallus is everted, the accessory apparatus is displaced at an angle of 45–90° from its uninflated position. In *Ips*, the endophallus is attached dorsally to the median lobe of the aedeagus and laterally to the ventral lobe, with both the median and ventral lobes limiting

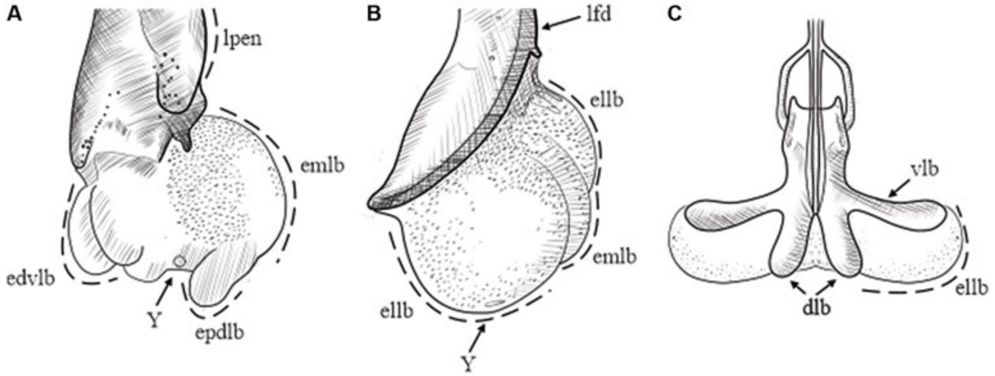

Figure 2. Inflated endophallus patterns in Scolytinae: **A**, *Dendroctonus* in lateral view; **B**, *Phloeosinus* in lateral view; and **C**, *Ips* in dorsal view. dlb, aedeagus' dorsal lobe; edvlb, endophallus' distal ventral lobes; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; epdlb, endophallus' proximal dorsal lobes; lfd, aedeagus' lateral folds; lpen, lateral lobes of pennis, Y, Y-structure; and vlb, aedeagus' ventral lobe. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

the movement of the endophallus during the eversion process, thereby giving it the characteristic of being retractile (Fig. 2C).

We found that the number of endophallus lobes differs among genera and species: *Dendroctonus* spp. have 3–5 lobes, *Phloeosinus* spp. have three lobes, and *Ips* spp. have two (Fig. 2). By comparing the relative position of the lobes, a common morphological pattern in endophalluses became apparent: the presence of a pair of lateral lobes in ventral and lateral views (Fig. 2), whose arrangement and shape differ among genera. The lateral lobes are oval and smaller than the median lobe in *Dendroctonus* but are rounded and as large or larger than the median lobe in *Phloeosinus* (Fig 2B); in *Ips*, only the lateral lobes are present, and their shape is uniform and oval (Fig. 2C). An additional pair of lobes – the proximal dorsal lobes and the distal ventral lobes – can be seen in lateral view in members of the *Dendroctonus frontalis* complex (Fig. 2A).

Similarities among genera were identified in the present study. In *Dendroctonus* and *Phloeosinus*, a median lobe and a yellow pore-like circular structure located in the posterior region of the median lobe are preserved (Figs. 4, 5, 6, 10, 12, 13, 15, and 16), whereas both structures are absent in *Ips* species (Fig. 2). Cerezke (1964, fig. 12) had previously identified the pore-like circular structure as the Y-structure in *D. ponderosae*. In *D. adjunctus*, *D. mesoamericanus*, *D. parallelocollis*, *D. pseudotsugae barragani*, and *D. valens* from Mexico, the Y-structure is poorly sclerotised; thus, it was not possible to obtain clear images that display this element (Fig. 3, 7, 8, 9, and 11). In the present study, spiny sclerite patterns are observed on the outer surface of the lobes in all species. Lateral lobes have evenly arranged conical spines. In *Dendroctonus,* the median lobe has smaller spines in closely spaced groups, with the spines becoming smaller towards the posterior end of the lobe, and no sclerotisation is observed on the lobe's ventral side.

***Dendroctonus*.** The endophallus is attached to the anchor arms and below the seminal rod. When pressure is exerted to evert the endophallus, the seminal rod and anchor move forwards, positioning themselves outside the capsule and forming an angle 45 and 90° from its initial position, in lateral view. The size of the endophallus varies among the species, although in *D. adjunctus, D. approximatus,* and *D. pseudotsugae barragani*, the internal sac is bigger than the aedeagus, and in *D. barberi, D. frontalis, D. mesoamericanus*, *D. parallelocollis*, and *D. vitei*, the internal is smaller than the aedeagus.

Details for each species are described below.

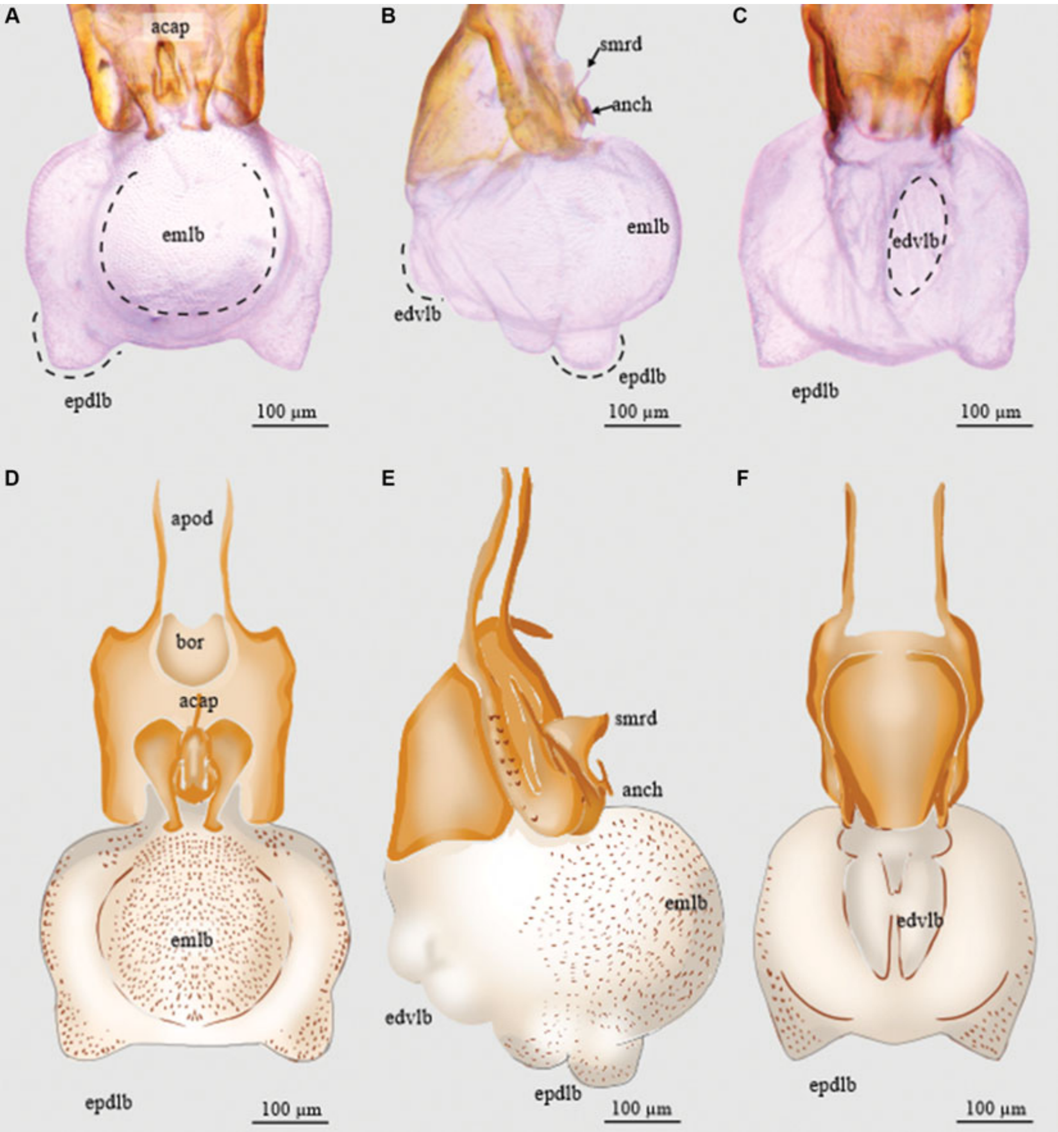

Figure 3. Endophallus of *Dendroctonus adjunctus*: **A** and **D**, dorsal view; **B** and **E**, lateral view; and **C** and **F**, ventral view. acap, accessory apparatus; anch, anchor; apod, apodemes; bor, basal orifice; edvlb, endophallus' distal ventral lobes; emlb, endophallus' median lobe; epdlb, endophallus' proximal dorsal lobes; and smrd, seminal rod. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

Dendroctonus adjunctus. The length of the endophallus (321 μm) is more than half the total length of the aedeagus (493 μm). The sac is composed of five lobes: one median lobe, two proximal dorsal lobes (Fig. 3A, D), and two distal ventral lobes (Fig. 3B, E). The median lobe is pronounced, with a rounded shape. The proximal dorsal lobes are small and are located on the apical region of the median lobe (Fig. 3C, F). The distal lobes are smaller than the dorsal proximal ones and are in the ventral region of the median lobe (3B, E). The Y-structure is located in the distal region of the sac (Fig. 3).

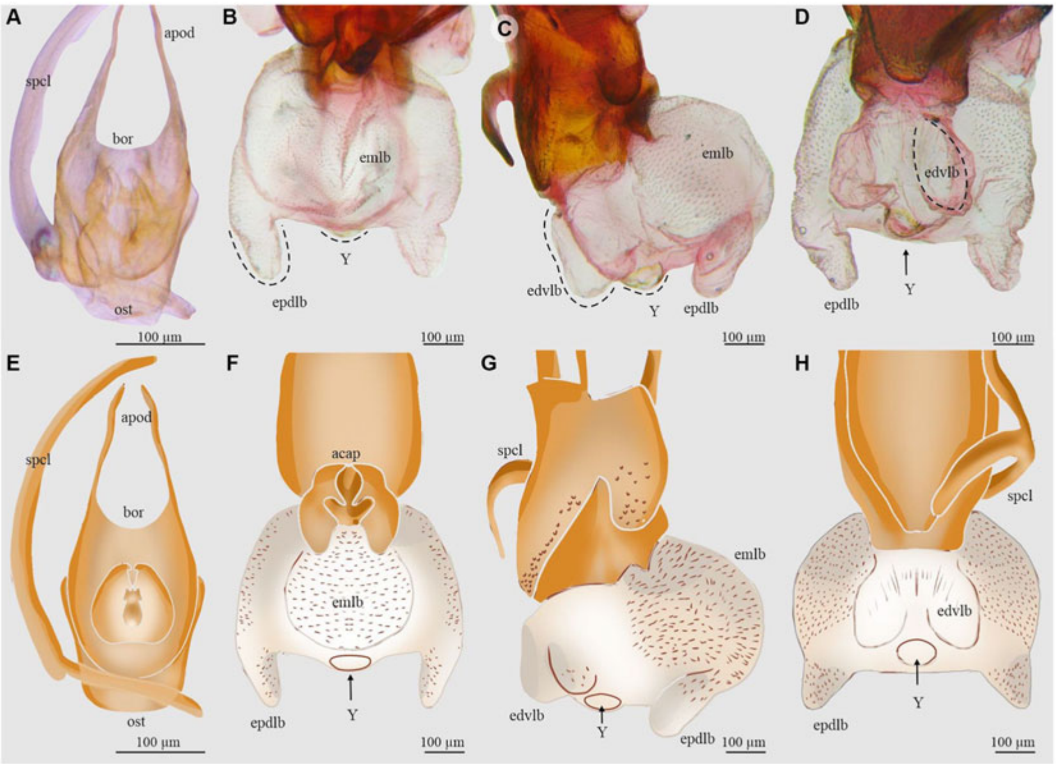

Figure 4. Aedeagus and endophallus of *Dendroctonus approximatus*: **A** and **E**, aedeagus in dorsal view; **B** and **F**, endophallus in dorsal view; **C** and **G**, endophallus in lateral view; **D** and **H**, endophallus in ventral view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; edvlb, endophallus' distal ventral lobes; emlb, endophallus' median lobe; epdlb, endophallus proximal dorsal lobes; ost, ostium; spcl, spicule; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

***Dendroctonus approximatus*.** The total length of the endophallus (449 µm) almost equals that of the aedeagus (581 µm; Fig. 4A, E). The endophallus is composed of five lobes: a prominent rounded median lobe in dorsal view (Fig. 4B, F), a pair of narrower and shorter distal lobes arising from the ventral surface of the median lobe (Fig. 4C, G), and a pair of proximal lobes arising from the dorsal surface of the median lobe (Fig. 4D, H). The Y-structure is located on the distal region of the sac (Fig. 4B, C, D, F, G, H).

***Dendroctonus barberi*.** The size of the endophallus (490 µm) is similar in proportion to that of the aedeagus (512.54 µm; Fig. 5A, D). It is formed by five lobes: a rounded median lobe in dorsal view (Fig. 5B, E), a pair of wider and larger proximal lobes arising from the ventral surface of the median lobe (Fig. 5C, F), and a pair of narrower, long distal lobes arising from the dorsal surface of the median lobe (Fig. 5B, C, E, F). The proximal and distal lobes are similar in shape, but the proximal lobes are larger than the distal lobes. In the posterior region in the central part of the median lobe, the Y-structure sits on a small evaginated surface of the endophallus (Fig. 5C, F).

***Dendroctonus frontalis*.** The endophallus (140 µm) is shorter than the aedeagus (297.03 µm; Fig. 6A, E). It consists of three lobes: an inconspicuous median lobe and two larger lateral lobes (Fig. 6B, F). In dorsal view, the sizes of the lateral lobes and the median lobe are similar in

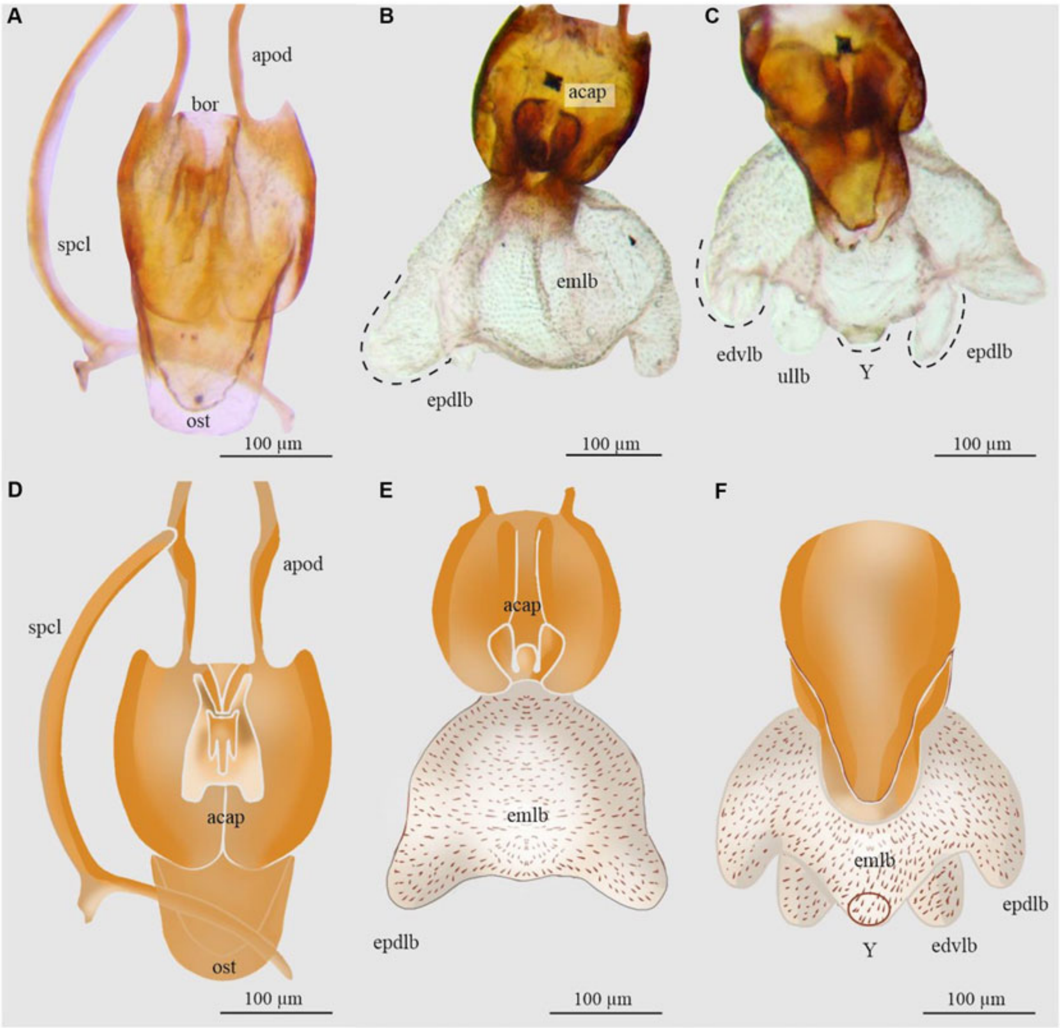

Figure 5. Aedeagus and endophallus of *Dendroctonus barberi*: **A** and **D**, aedeagus in dorsal view; **B** and **E**, endophallus in dorsal view; **C** and **F**, endophallus in ventral view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; edvlb, endophallus' distal ventral lobes; emlb, endophallus' median lobe; ost, ostium; epdlb, endophallus' proximal dorsal lobes; ost, ostium; spcl, spicule; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

proportion. The median lobe in the distal region presents the Y-structure (Fig. 6B, C, D, F, G, H). The lateral lobes are covered with small spines evenly distributed over the entire outer surface (Fig. 6C, G).

***Dendroctonus mesoamericanus*.** The length of the endophallus (181 µm) is similar to that of the capsule (172 µm; Fig. 7A, D). It consists of three lobes: a conspicuous median lobe and two well-defined lateral lobes (Fig. 7B, E). The median lobe is bigger than the lateral ones. The Y-structure is in the distal region of the endophallus, between lateral lobes. The surface of the median lobe is covered by large, evenly distributed conical spines, and the lateral lobes are covered by smaller spines (Fig. 7C, F).

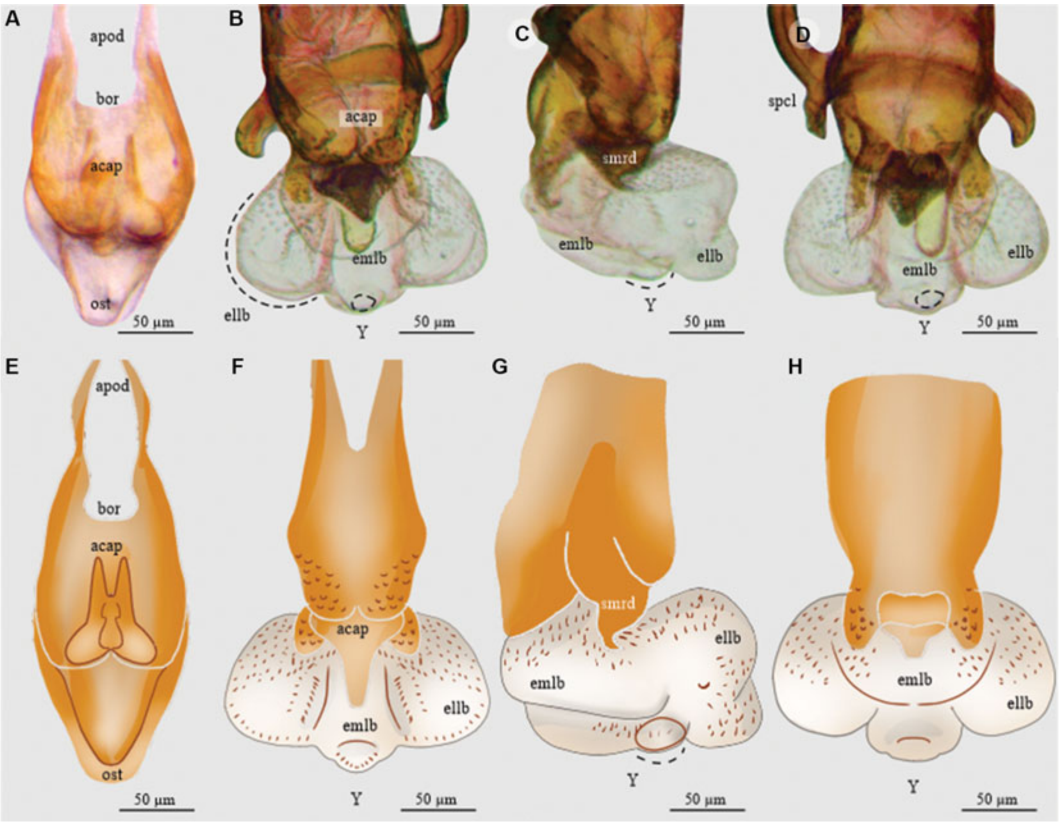

Figure 6. Aedeagus and endophallus of *Dendroctonus frontalis*: **A** and **E**, aedeagus in dorsal view; **B** and **F**, endophallus in dorsal view; **C** and **G**, endophallus in lateral view; and **D** and **H**, endophallus in ventral view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; ost, ostium; smrd, seminal rod; spcl, spicule; and Y, Y-structure. (See Supplementary material, abbreviations S1 for a list and definitions of the abbreviations.)

Dendroctonus parallelocollis. The length of the endophallus (307 µm) is proportional to that of the capsule (582 µm). It consists of three lobes: a well-defined median lobe in dorsal and lateral views (Fig. 8A, B) and two distal lateral lobes that extend perpendicular to the median lobe and arise from the sides of the posterior region of the median lobe (Fig. 8B, E). The shape of the lateral lobes differs from that of the median lobe because it has some folds that give it an irregular shape that is widest at the base of the lobes (Fig. 8C, F). The Y-structure is in the posterior region of the median lobe. Small conical spines are distributed on the dorsal surface of the median lobe and the anterior region of the lateral lobes, on the median lobe, the density of spines decreases towards the lateral lobes (Fig. 8B, D, E, F).

Dendroctonus pseudotsugae barragani. The length of the endophallus in dorsal view (382 µm) is almost half the total length of the aedeagus (774 µm; Fig. 9A, C). The endophallus of this species consists of three lobes that are poorly differentiated: a prominent median lobe and two small distal lateral lobes (Fig. 9B, D). The surface of the median lobe covers almost the entire endophallus. In the dorsal region, the median lobe is covered by small spines, the density of which decreases towards the lateral lobes (Fig. 9D). The Y-structure is found in the ventral posterior region of the median lobe.

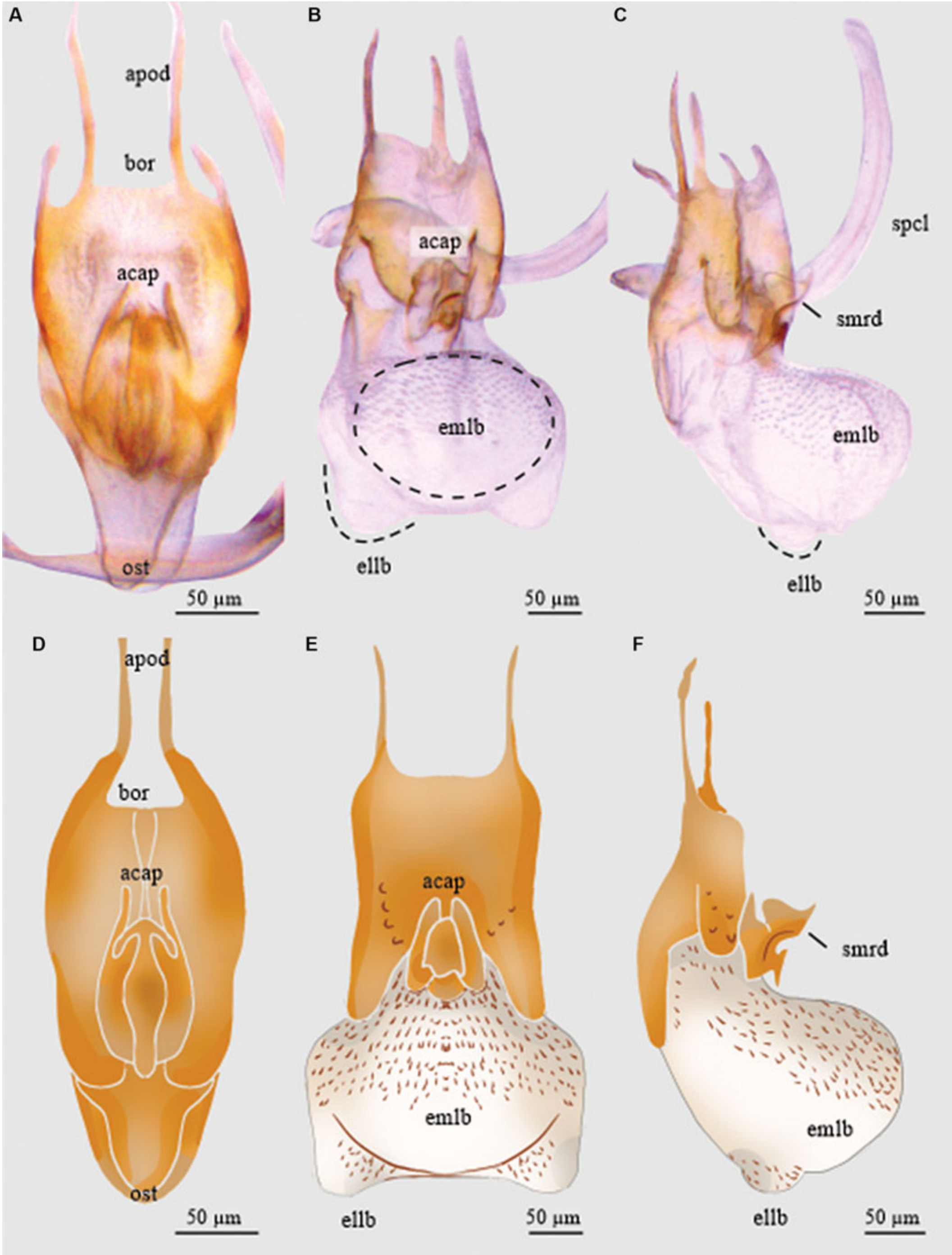

Figure 7. Aedeagus and endophallus of *Dendroctonus mesoamericanus*: **A** and **D**, aedeagus in dorsal view; **B** and **E**, endophallus in dorsal view; and **C** and **F**, endophallus in lateral view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; ost, ostium; smrd, seminal rod; and spcl, spicule (see Supplementary material, File S1 for a list and definitions of the abbreviations)

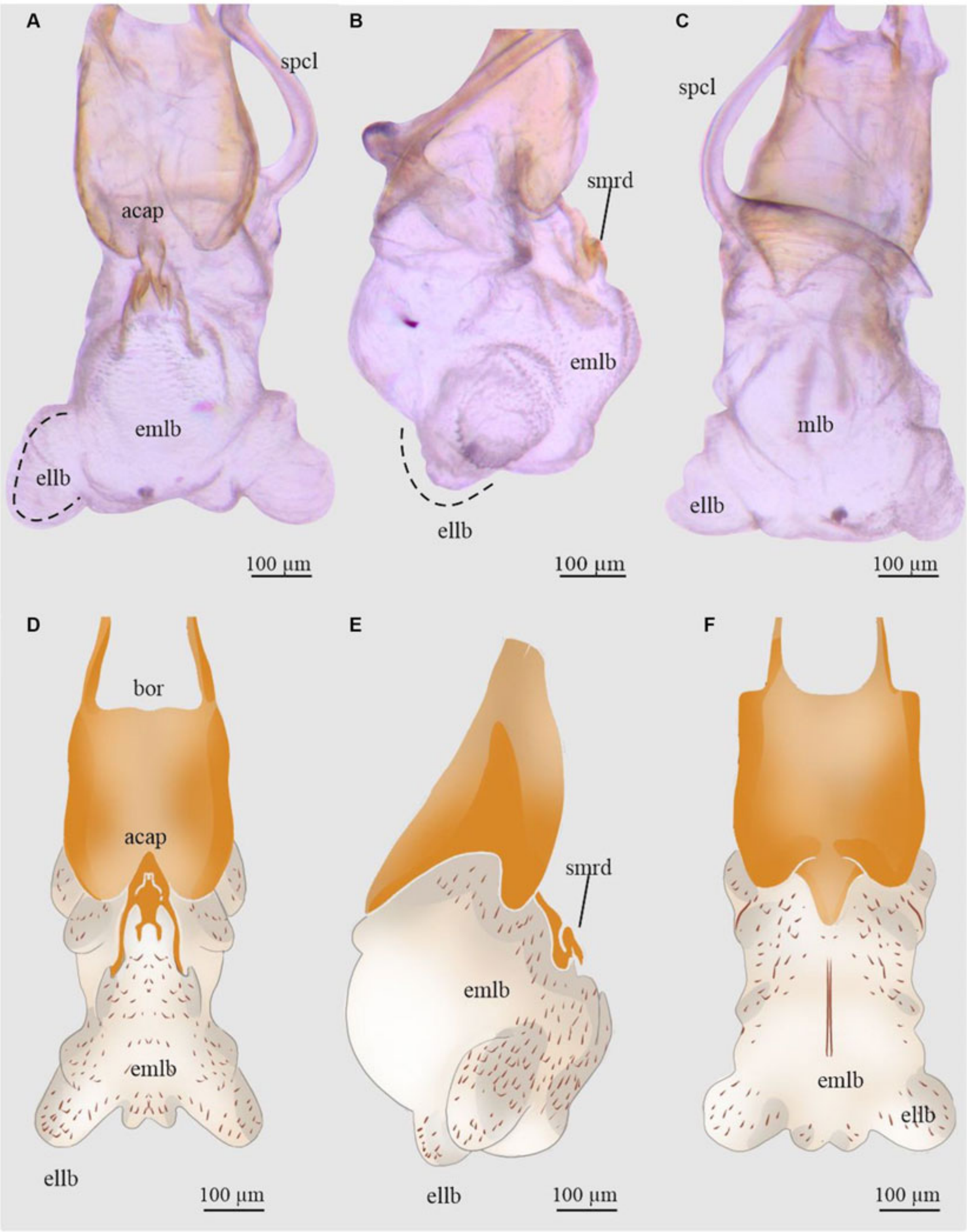

Figure 8. Endophallus of *Dendroctonus parallelocollis*: **A** and **D**, aedeagus in dorsal view; **B** and **E**, endophallus in lateral view; and **C** and **F**, endophallus in ventral view. acap, accessory apparatus; bor, basal orifice; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; smrd, seminal rod; and spcl, spicule. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

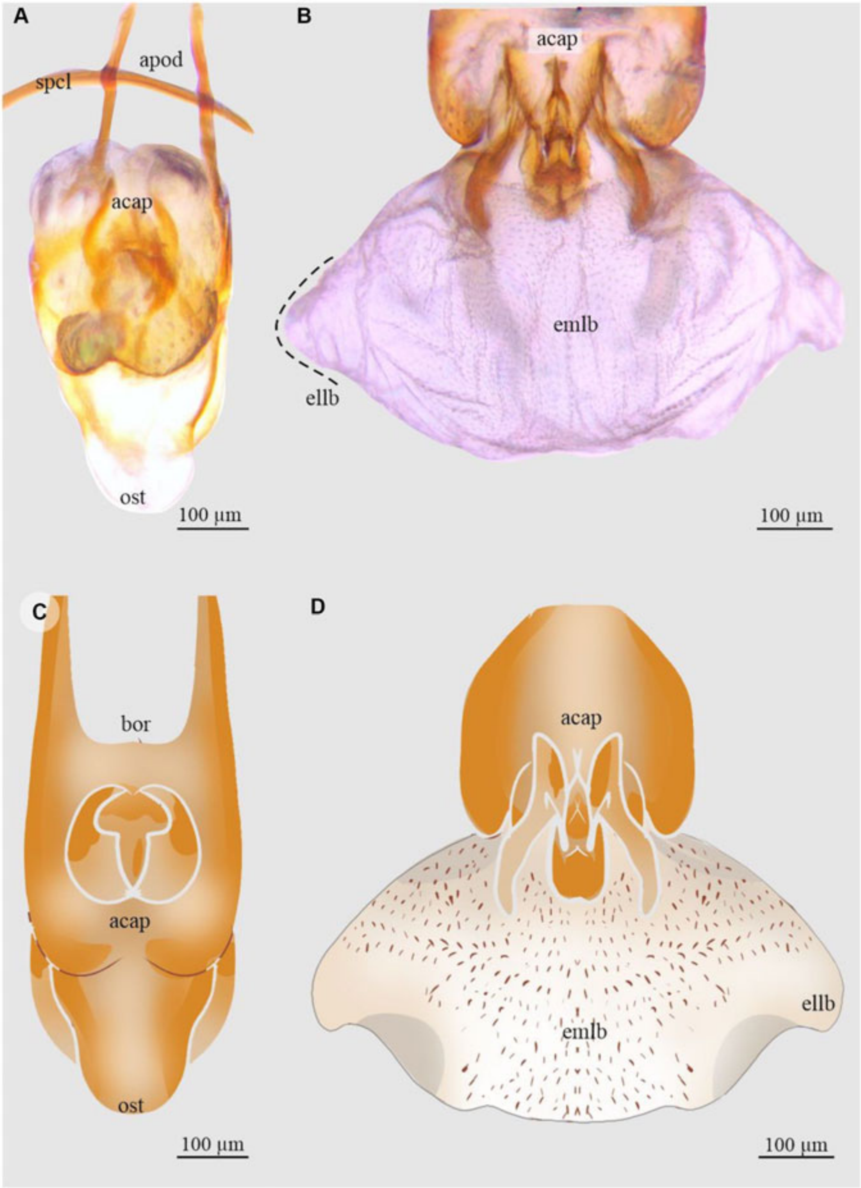

Figure 9. Aedeagus and endophallus of *Dendroctonus pseudotsugae barragani*: **A** and **C**, aedeagus in dorsal view; and **B** and **D**, endophallus in dorsal view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; ellb, endophallus' lateral lobe; emlb, endophallus' median lobe; ost, ostium; and spcl, spicule. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

Dendroctonus rhizophagus. A partial inflation of the endophallus was obtained with these specimens. In dorsal view, the endophallus was found to be formed by at least three lobes that resemble a "T" shape (Fig 10A): a poorly developed median lobe and two well-defined distal lateral lobes (Fig. 10A, C). The median lobe is larger than the lateral lobes. The Y-structure sits between the two distal lateral lobes, on the distal region of the endophallus (Fig. 10B, D).

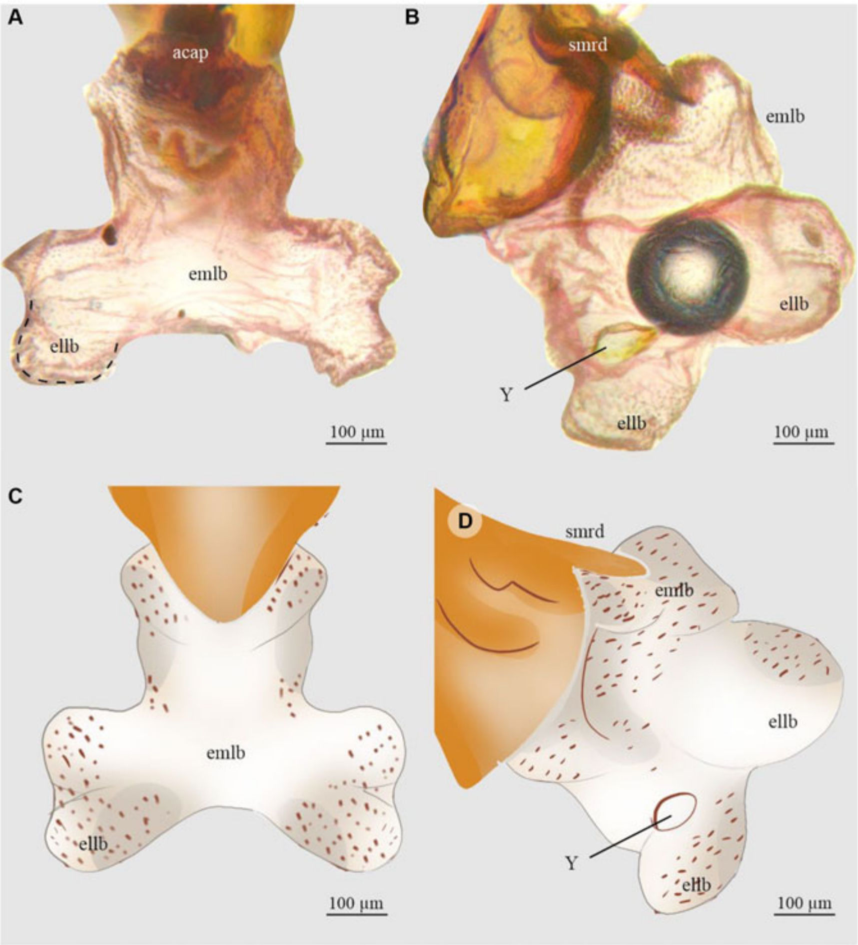

Figure 10. Endophallus of *Dendroctonus rhizophagus*: **A** and **C**, dorsal view; **B** and **D**, lateral view displaying the median lobe and the lateral lobes. acap, accessory apparatus; ellb, endophallus' lateral lobes lobes; emlb, endophallus' median lobe; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

***Dendroctonus valens* from Mexico.** The length of the endophallus is almost proportional to the capsule of the aedeagus (655 µm) and is formed by three lobes that resemble a "Y" shape in ventral view: an inconspicuous median lobe and two prominent distal lateral lobes (Fig. 11A, D). The median lobe is poorly differentiated from the lateral lobes (Fig. 11B, E). Each apex of the lateral lobes ends in a point. The dorsal and lateral surfaces of the endophallus are covered by small spines. The Y-structure sits between the two distal lateral lobes, in the distal region of the endophallus. The density of the spines decreases to the ventral region (Fig. 11D, E, F).

***Dendroctonus valens* from Honduras.** The endophallus is similar to those of *D. rhizophagus*, formed by three lobes that resemble a short "T" shape in ventral view: a conspicuous median lobe that is divided into two well-defined regions (distal and proximal; Fig. 12A, D); the proximal region is rounded in dorsal view, and the distal region is fused with the two lateral lobes. The apical edge of each lateral lobe is divided into two small lobes that are equal in size and shape, both being rounded (Fig. 12B, E). The median lobe is equal in length to the lateral lobes. The Y-structure sits between the two distal lateral lobes, in the distal region of the endophallus

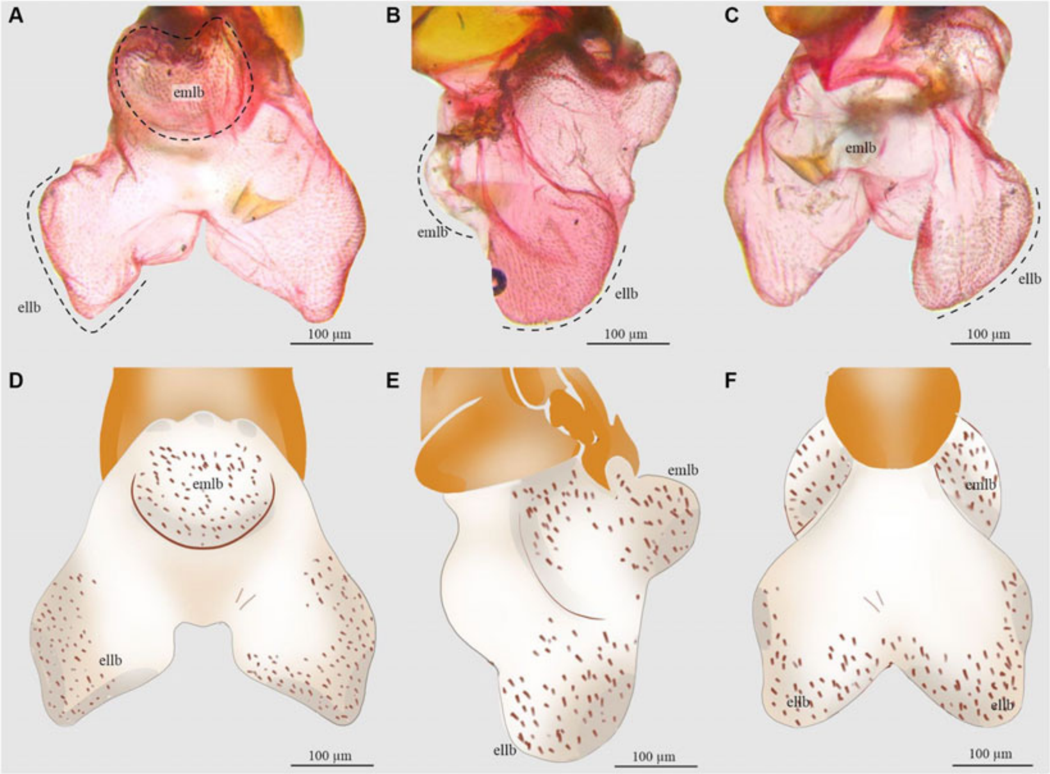

Figure 11. Aedeagus of *Dendroctonus valens* from Mexico: **A** and **D**, dorsal view; **B** and **E**, lateral view; and **C** and **F**, ventral view. ellb, endophallus' lateral lobes lobes; and emlb, endophallus' median lobe. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

(Fig. 12C, F). The entire surface of the endophallus is covered by small spines, which decrease in density towards the ventral region (Fig. 12D, E, F).

***Dendroctonus vitei*.** The length of the endophallus (208 µm) is more than a half total length of the aedeagus (367 µm). The endophallus is formed by three lobes: a prominent rounded median lobe in dorsal view (Fig. 13A) and two lateral distal lobes that, in dorsal view, are small, very rounded, and are as long as wide (Fig. 13A, D). The lateral lobes are smaller than the median one (Fig. 13B, E). The Y-structure is located in the posterior ventral region of the median lobe (Fig. 13C, F). The entire surface of the endophallus is covered by small spines which decrease in density from the centre of the dorsal region of the median lobe towards the ventral region (Fig. 13D, E, F).

***Phloeosinus*.** The endophallus in this genus is a thin membranous sac, the base of which is attached to the accessory apparatus and the dorsal–distal region of the ostium. The sac surrounds the seminal rod and is fused with the anchor, which is located above the seminal rod (Fig. 14A, B, C, D, E). When the endophallus is everted or inflated, the seminal rod performs a 45° movement. In general, the endophallus of *Phloeosinus* is formed by three lobes: a median lobe and two lateral lobes that have shapes that are quite uniform in all the species analysed. In the sampled species, the size of the internal sac is similar and not bigger than seminal capsule. In addition, the Y-structure is located in the posterior region of the median lobe and, in lateral view, has a conical shape.

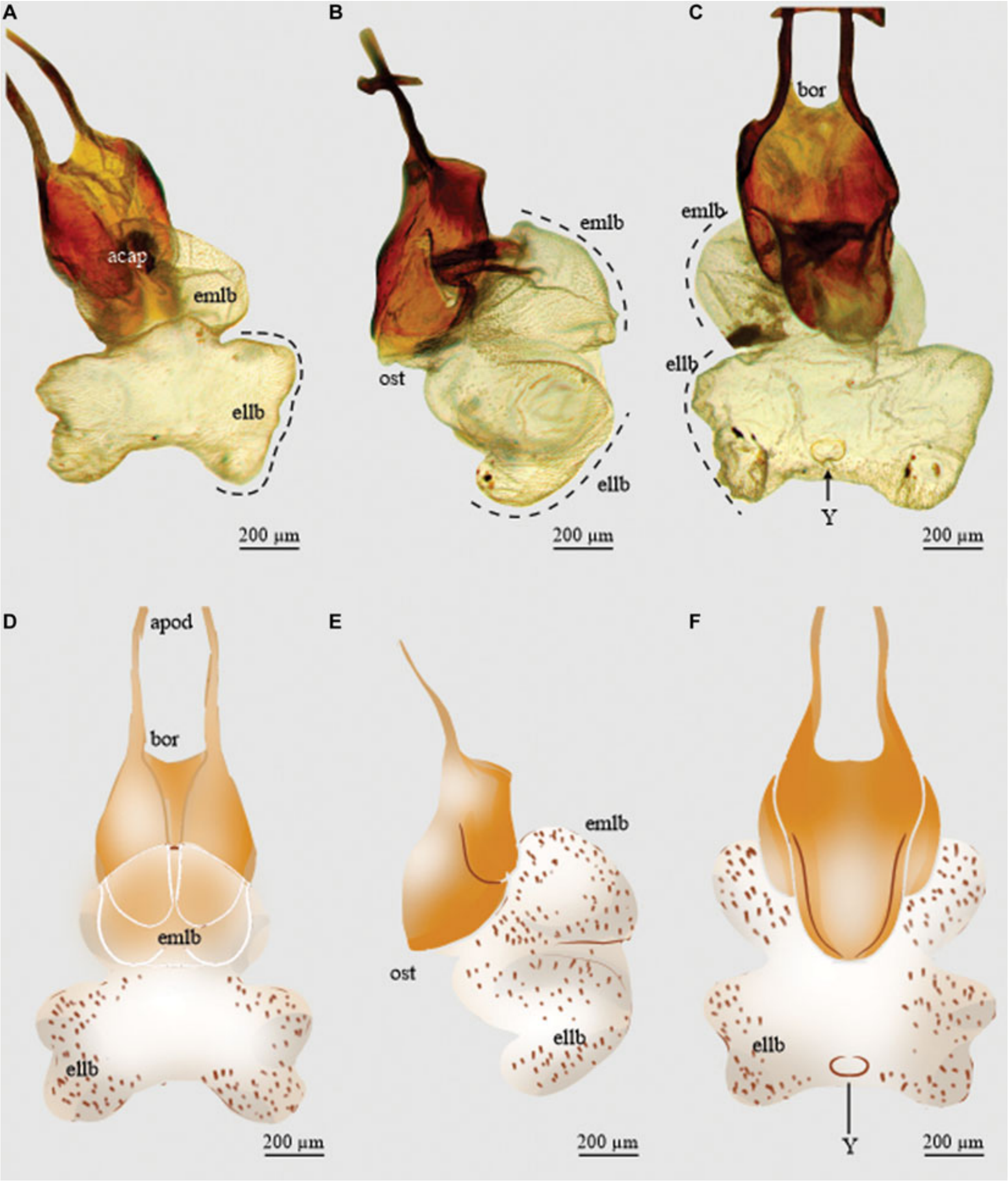

Figure 12. Endophallus of *Dendroctonus valens* from Honduras: **A** and **D**, dorsal view; **B** and **E**, lateral view; and **C** and **F**, ventral view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; ost, ostium; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

***Phloeosinus baumanni*.** The length of the endophallus in the dorsal view (280 µm) is approximately one-half the total length of the aedeagus (542 µm). The sac consists of a conspicuous median lobe that extends from the basal orifice connected to the accessory apparatus to the ostium (Fig. 15A) and of a pair of poorly marked lateral lobes that protrude further than the median lobe and expand beyond the ostium (apical region of the aedeagus). The shape of the

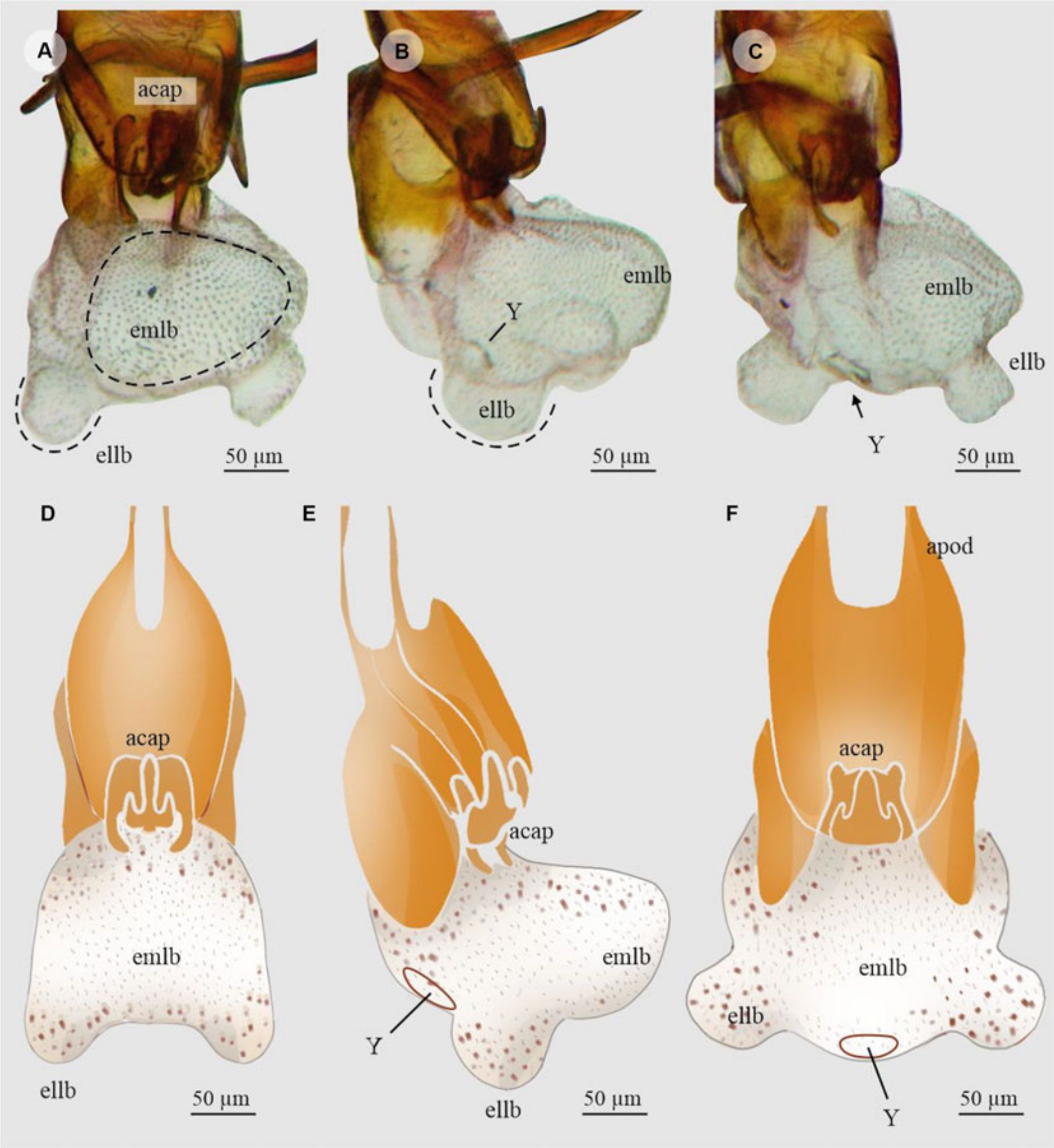

Figure 13. Aedeagus and endophallus of *Dendroctonus vitei*: **A** and **D**, dorsal view; **B** and **E**, lateral view; and **C** and **F**, ventral view. acap, accessory apparatus; apod, apodemes; ellb, endophallus' lateral lobe; emlb, endophallus' median lobe; and Y, Y-structure. (See Supplementary material, abbreviations S1 for a list and definitions of the abbreviations.)

endophallus lobes is rounded (Fig. 15B, E). The lateral lobes are not well differentiated from the median lobe, and they appear to be the same size as the median lobe in lateral view (Fig. 15A, D). All lobes are covered by small spines which are denser at the base (anterior region) near the accessory apparatus (Fig. 15C). The Y-structure is located in the posterior region of the median lobe (Fig. 15D, E, F).

***Phloeosinus deleoni*.** The length of the endophallus (328.58 µm) is more than half of the total length of the aedeagus (537.35 µm) in dorsal view (Fig.16A). The median lobe is prominent compared to the lateral lobes (Fig. 16B, E). In the posterior region, the median lobe is rounded,

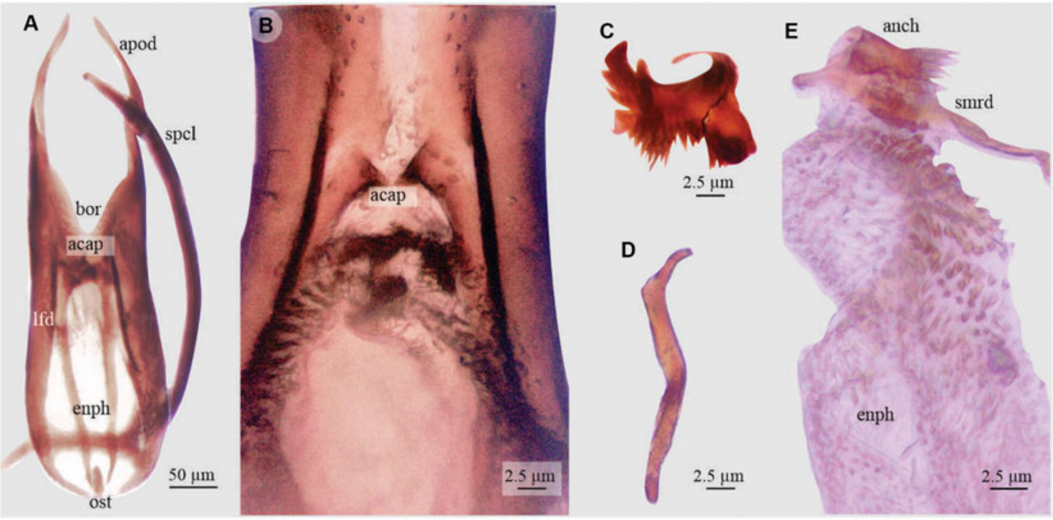

Figure 14. The structures that conform to the genital organ of *Phloeosinus*: **A** and **B**, aedeagus in dorsal view; **C**, anchor lateral view; **D**, seminal rod lateral view; and **E**, endophallus. acap, ccessory apparatus; anch, anchor, apod, apodemes; bor, basal orifice; enph, endophallus; lfd, aedeagus' lateral folds; ost, ostium; spcl, spicule; and smrd, seminal rod. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

and the lateral lobes display oval edges (Fig. 16C, F). The Y-structure is located in the median lobe apex (Fig. 16B, C, E, F). All lobes have spines in their dorsal regions; the spines are denser at the lobes' bases (next to the accessory apparatus) and decrease in density towards the anterior regions (Fig. 16D, E).

Phloeosinus tacubayae. The length of the endophallus (294.33 µm) is approximately three-quarters of the total length of the aedeagus (441.09 µm) in dorsal view (Fig. 17A). The endophallus consists of three inconspicuous lobes (Fig. 17B, D). The median lobe is prominent compared to the lateral lobes and is rounded in the anterior region (Fig. 17C, E). The two lateral lobes are poorly differentiated from the median lobe and are much less evaginated than the median lobe – in some views, the endophallus appears to be unilobate (Fig. 17B–E). In the apex of the median lobe is a protruding Y-structure (Fig. 17C, D, E). Spines cover the anterior region near the base of the endophallus, which become less dense towards the posterior–apical region (Fig. 17B–E).

Phloeosinus serratus. For this species, specimens from three populations collected from different Cupressaceae host plants, *Hesperocyparis arizonica* (Miller) Bartel, *Juniperus coahuilensis* (Martínez) Gaussen ex. R.P. Adams, and *Juniperus saltillensis* M.T. Hall, were studied. Specimens from the three hosts present conspicuous morphological differences in external and female genital morphology; however, of the three populations of *P. serratus*, the characteristics of the endophallus were similar (Fig. 18): the endophallus consists of a median lobe and two smaller lateral lobes. In the region near the accessory apparatus (Fig. 18B–G), the surface of the endophallus is covered by small spines, which decrease in density towards the sides (Fig 18G). The Y-structure is in the apical and central region of the endophallus and protrudes from the median lobe (Fig. 18H, I).

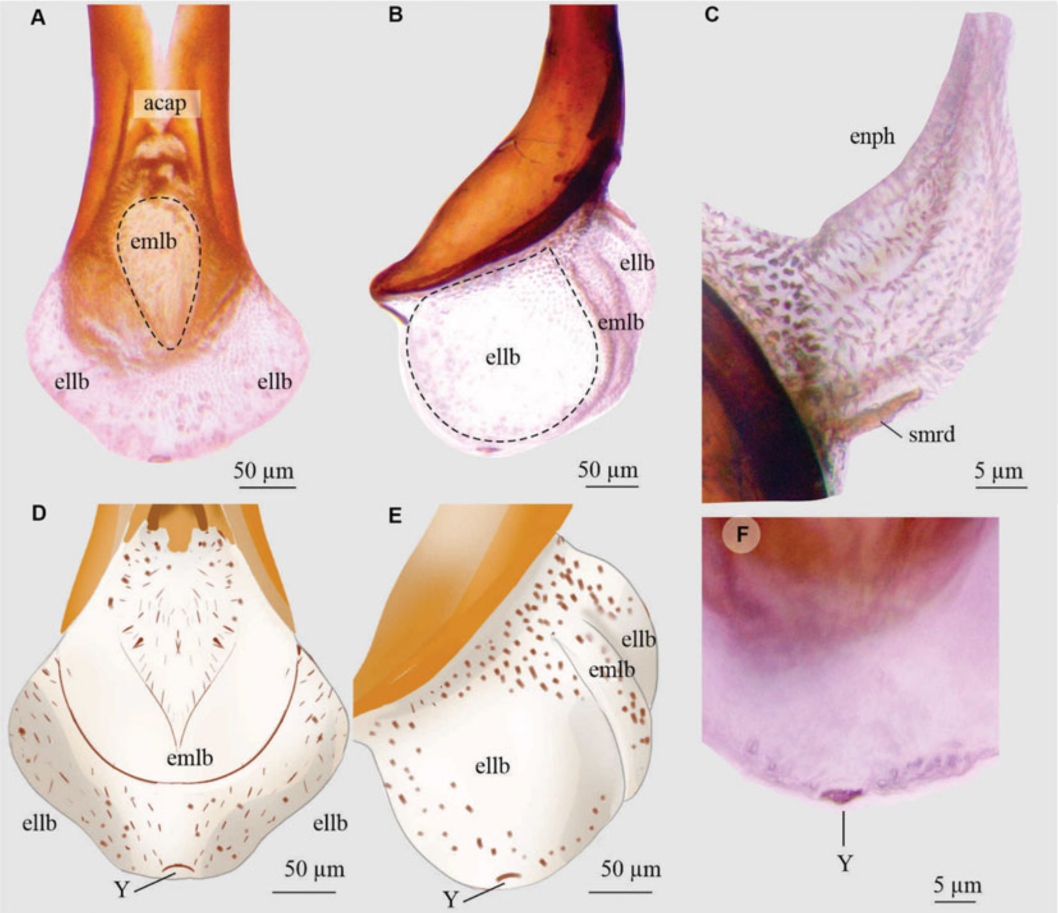

Figure 15. Aedeagus and endophallus of *Phloeosinus baumanni*: **A** and **D**, aedeagus in dorsal view; **B** and **E**, endophallus in lateral view, **C**, accessory apparatus in lateral view, and **F**, Y structure in lateral view. acap, accessory apparatus; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; enph, endophallus; smrd, seminal rod; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

Ips. The endophallus is a retractile eversible sac and has two lateral lobes. The sac is attached to the dorsal lobes of the aedeagus in the median part of the dorsal region (junction point of both lobes) and is joined on the sides with the ventral lobes (Figs. 19, 20, and 21). The seminal trough is located in the central part of the aedeagus and, at the time of inflation, descends slightly towards the posterior part of the endophallus (Fig. 19A). When inflation is performed, the ventral lobes expand sideways, allowing the eversion of the endophallus: the movement resembles the opening of a fan (Figs. 20B and 21D, E). However, when the pressure exerted during inflation is removed, the endophallus retracts, and the ventral lobes return to their initial positions. In the two species analysed, the size of the lobes is proportional to the length of the ventral lobes of the aedeagus.

Ips calligraphus. In this species, when the endophallus is inflated and everted, the ventral and dorsal lobes of aedeagus are slightly moved together with the endophallus lobes (Fig. 20D, E). The point of the ventral lobe expands poorly to the sides, forming an angle of less than 45° to the dorsal lobes, and the dorsal lobes are slightly separated (Fig. 20B, C). The endophallus lateral lobes are as long as wide as and longer than the aedeagus lobes (Fig. 20D, E).

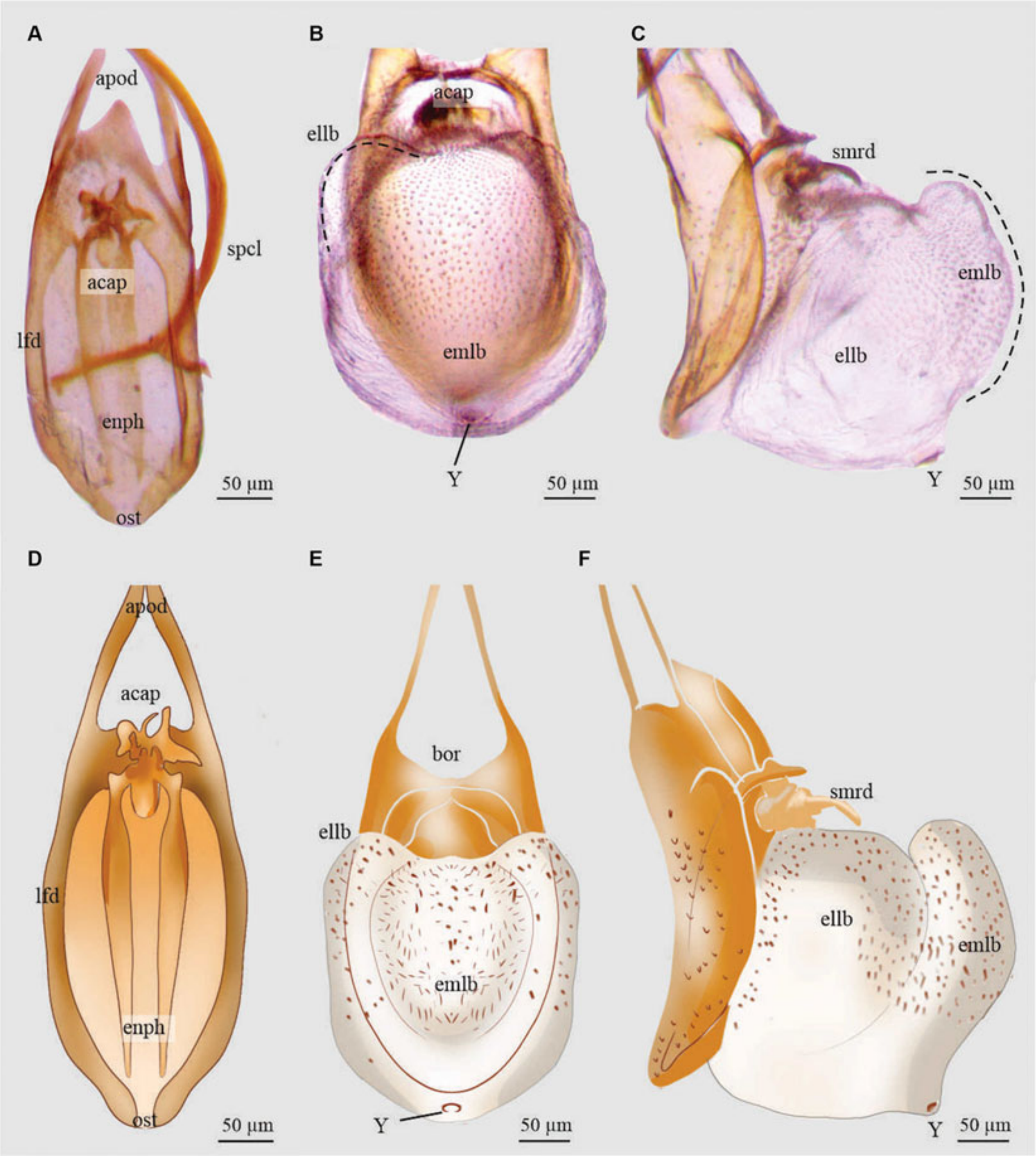

Figure 16. Aedeagus and endophallus of *Phloeosinus deleoni*: **A** and **D**, aedeagus in dorsal view; **B** and **E**, endophallus dorsal view; and **C** and **F**, endophallus in lateral view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; enph, endophallus; ellb, endophallus' lateral lobes; lfd, aedeagus' lateral folds; emlb, endophallus median lobe; smrd, seminal rod; spcl, spicule; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

Ips lecontei. When the endophallus is inflated and everted in this species, the ventral and dorsal lobes of aedeagus are strongly moved together with the endophallus lobes (Fig. 21B). The ventral lobe expands sideways, forming a 90° angle to the dorsal lobes, and the dorsal lobes separate slightly between them (Fig. 21B). The endophallus lateral lobes are longer than wide, their length is slightly higher than the ventral lobe, and their width is equivalent to dorsal lobe length (Fig. 21B).

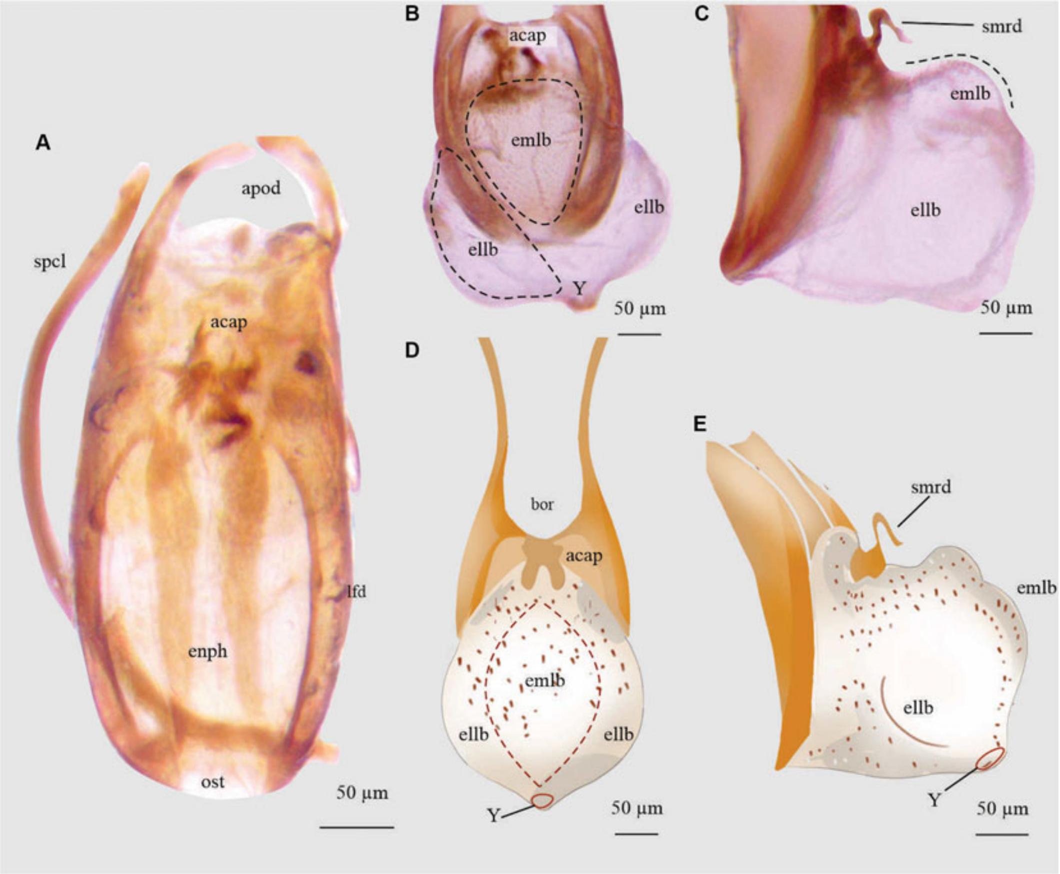

Figure 17. Aedeagus and endophallus of *Phloeosinus tacubayae*: **A**, aedeagus dorsal view; **B** and **D**, endophallus dorsal view; and **C** and **E**, endophallus lateral view. acap, accessory apparatus; apod, apodemes; bor, basal orifice; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; enph, endophallus; lfd, aedeagus' lateral folds; smrd, seminal rod; spcl, spicule; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

Discussion

In this study, Van Dam's (2014) technique was modified for the study of the endophallus of Scolytinae, allowing the description of the morphology of the internal sac and the inflation patterns in 16 species from *Dendroctonus*, *Ips*, and *Phloeosinus*. The species differ in endophallus attachment types and display two distinct inflation and retraction mechanisms. Our results show that the internal sac is a potentially useful tool for Scolytinae taxonomy, as each genus appears to have its own morphological patterns, particularly in *Dendroctonus*, where differences and similarities are more pronounced in closely related species.

Previous studies that use the endophallus generated several techniques to facilitate the organ's processing and eversion and to allow its conservation once inflated (Berlov 1992; Uliana and Sabatinelli 2010; Janovska *et al.* 2013; Daccordi *et al.* 2020). An important issue with those methods, including the most widely used technique, the Berti–Vachon method (Bontems 2013), is their applicability in small and pin-mounted specimens. Van Dam (2014) presented an easy technique for endophallus eversion that is fast and can be used in various taxa within Curculionidae. The modifications to this technique that were tested in the present study, including the rehydration of dry specimens, the two digestion steps (enzyme and KOH), the dilution of

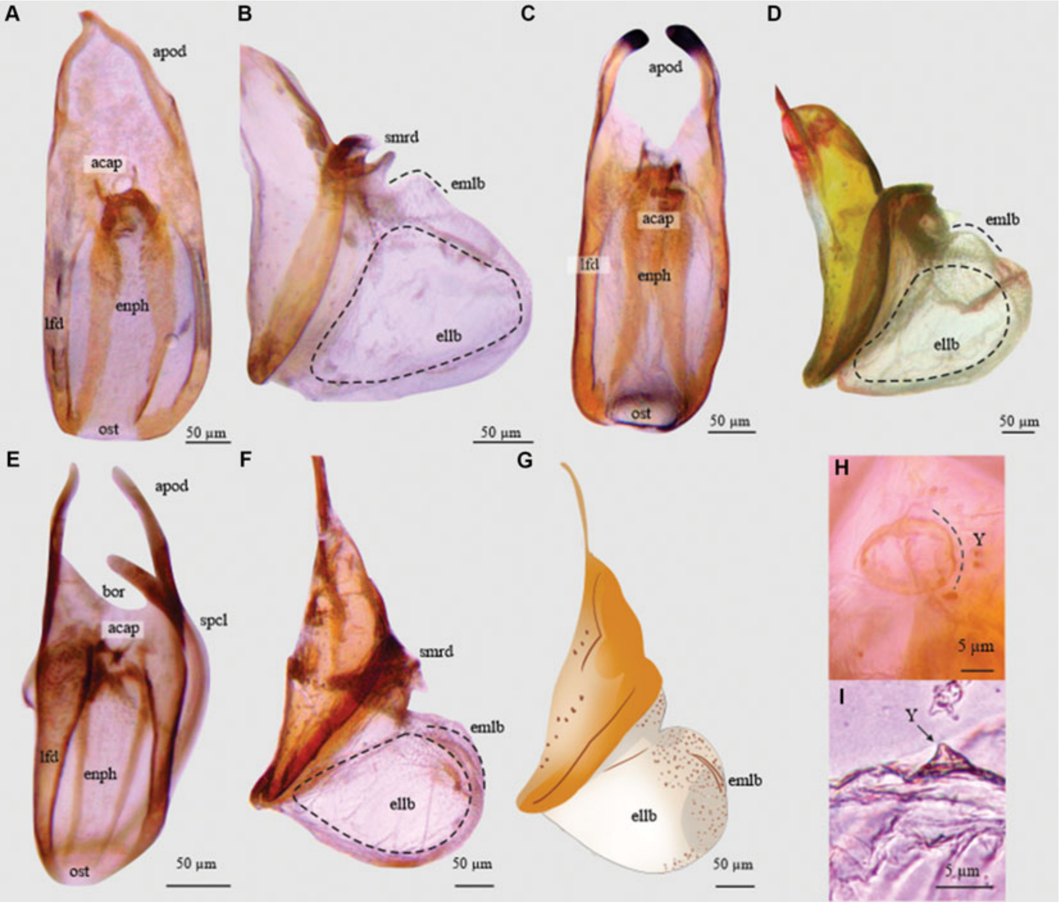

Figure 18. Aedeagus and endophallus of *Phloeosinus serratus*: **A**, **C**, and **E**, aedeagus dorsal view; **B**, **D**, **F**, and **G**, endophallus lateral view; **H** and **I**, Y-structure in dorsal and lateral view, respectively. Specimens from *Hesperocyparis arizonica* (**A**, **B**); *Juniperus coahuilensis* (**C**, **D**); and *J. saltillensis* (**E**, **F**, and **G**). acap, accessory apparatus; apod, apodemes; bor, basal orifice; ellb, endophallus' lateral lobes; emlb, endophallus' median lobe; enph, endophallus; lfd, aedeagus' lateral folds; ost, ostium; smrd, seminal rod; spcl, spicule; and Y, Y-structure. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

inflation fluid, and the modifications of the tools (forceps and syringes), together enabled satisfactory results with Scolytinae specimens that were preserved in different ways. First, we were able to inflate the endophalluses of large species, such as *D. rhizophagus* (5.0–6.3 mm) and *D. valens* (5.3–8.3 mm), and small ones, such as *D. frontalis* (2.0–3.2 mm) and *P. tacubayae* (1.9–2.4 mm), which represent a range in body size among the family. Also, the technique worked properly in both fresh and pin-mounted specimens and permitted relatively fast endophallus inflation, facilitating the analysis of at least two specimens per species.

Two techniques have been implemented in other curculionids for endophallus eversion. The first is the Berti–Vachon (Bollino and Sandel 2017; Meregalli *et al.* 2020), and the second and more recent is the Van Dam (2014) technique. Both consist of three general steps: (1) the dissection and enzymatic digestion of the aedeagus, (2) the inflation of the endophallus, and (3) the preparation of the endophallus for photography. In Scolytinae specimens, these three steps permit relatively good results in fresh specimens; however, in specimens that have been preserved in alcohol for several years and in those that are dry-mounted, muscle tissues are hard, and

Figure 19. Complete genital organ in dorsal view of *Ips*: **A**, aedeagus; **B**, spicula; **C**, tegmen; **D**, seminal trough, **E**, retracted endophallus in dorsal view, and **F,** retracted endophallus in ventral view. apod, apodemes; dlb, aedeagus' dorsal lobe; enph, endophallus; smt, seminal trough; and vlb, aedeagus' ventral lobe. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

dissecting without damaging the abdominal segments and aedeagus pieces is difficult. As our results show, including a prior rehydration step that uses a "brine" softening solution softens the cuticular membranes and muscle fibres and allows cleaner dissections and isolation of the aedeagus with greater ease and integrity.

In other curculionids, the pancreatin enzymatic solution by itself digests tissues within a couple of hours (Van Dam 2014). However, our results indicate that, in dry-preserved specimens or those with highly chitinised structures – such as *D. adjunctus, D. approximatus, D. parallelocollis, D. rhizophagus, D. valens*, and *P. baumanni* – the pancreatin solution alone is insufficient for soft tissue degradation and that adding a second digestion step consisting of a low concentration

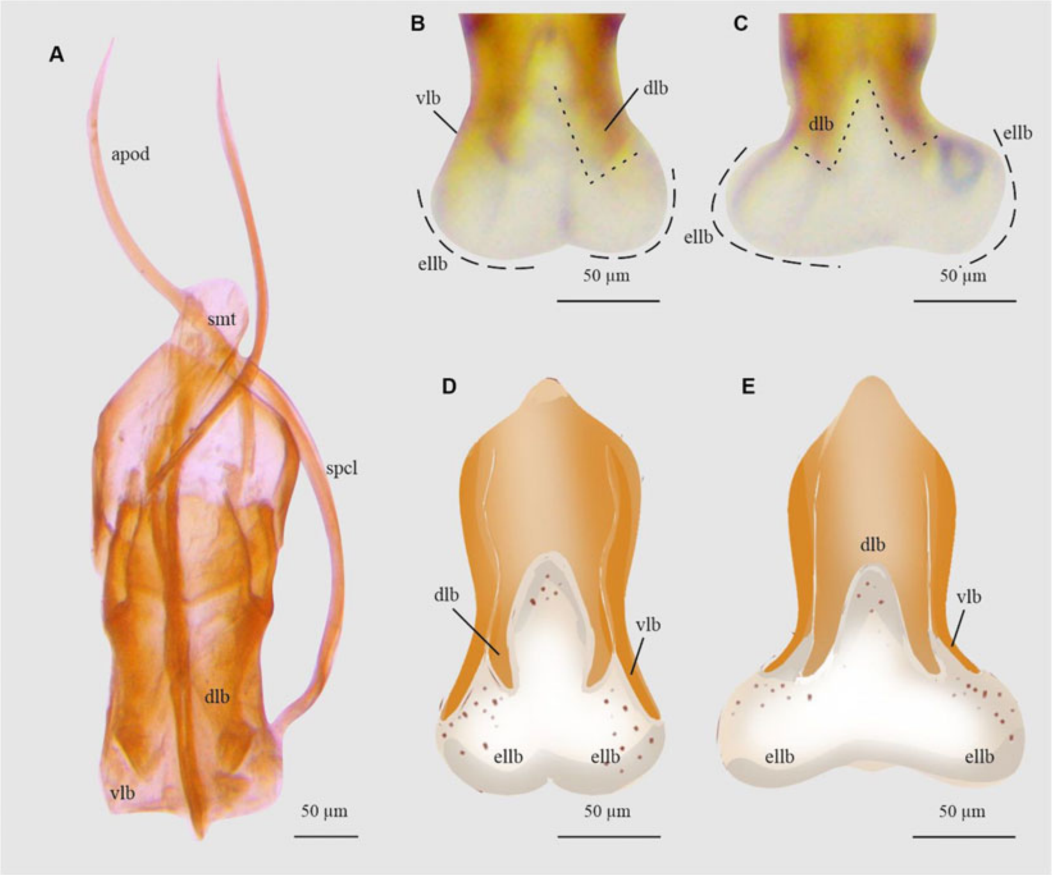

Figure 20. Aedeagus and endophallus of *Ips calligraphus*: **A**, aedeagus dorsal view; **B** and **D**, endophallus dorsal view; and **C** and **E**, endophallus ventral view. apod, apodemes; dlb, aedeagus' dorsal lobe; ellb, endophallus' lateral lobes; smt, seminal trough; spcl, spicule; and vlb, aedeagus' ventral lobe. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

solution of KOH is required. The use of K-Y gel, which is transparent, as a filling substance allows the details of the endophallus to be observed, unlike the use of opaque substances such as toothpaste (Uliana and Sabatinelli 2010; Janovska *et al.* 2013), which mask the endophallus's sclerotised structures. However, we found it necessary to dilute the gel (50%) because the viscosity provided by the manufacturer prevented the gel from flowing through and inflating the structures. Despite the decreased viscosity, the gel strengthened the sampled tissues and allowed their manipulation with a low risk of collapse, as can happen when other fluids such as alcohol and glycerin are used (Yamasako and Ohbayashi 2011).

One of the key points to achieving successful endophallus inflation is a good coupling and clamping of the base of the aedeagus with the forceps. Van Dam (2014) suggests using microvascular corneal forceps (no. 18155-13); however, the size of the aedeagus in Scolytinae is small, and these forceps are coarse and stiff, which hinders the manipulation of the aedeagus and therefore the inflation process. In the case of very small specimens such as Scolytinae, we recommend using very fine-tipped forceps because this allows better control when manipulating the sample during the inflation process. Also, we recommend using 70% alcohol to preserve the inflated samples: even though the duration of preservation is not yet defined, the method seems to be efficient.

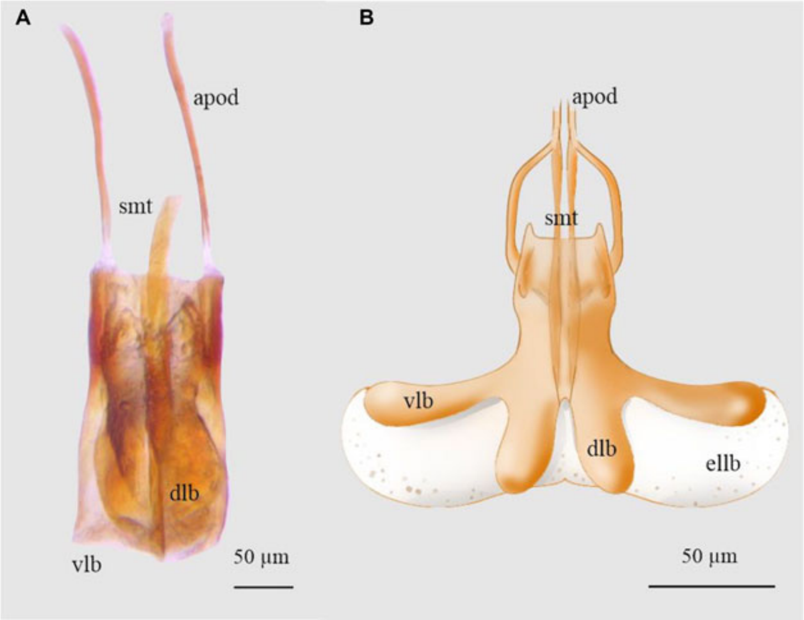

Figure 21. Aedeagus and endophallus of *Ips lecontei* in dorsal view: **A**, aedeagus; **B**, endophallus. apod, apodemes; dlb, aedeagus' dorsal lobe; ellb, endophallus' lateral lobes; smt, seminal trough; and vlb, aedeagus' ventral lobe. (See Supplementary material, File S1 for a list and definitions of the abbreviations.)

Anatomy and nomenclature

Prior studies on the anatomy of the endophallus in the Coleoptera have focused mainly on descriptions of the organ's lobes and sclerotised structures, especially in related or individual species (Bollino and Ruzzante 2015; Bollino and Sandel 2017; Meregalli *et al.* 2020). Génier (2019) proposes standardising the nomenclature of several sclerosed elements of the insect endophallus; however, the terms used to name and describe the aedeagal lobes remain unhomogenised mainly because the lobes' numbers, positions, and shapes display great intra- and interspecific variation among taxa (Bollino and Ruzzante 2015; Bollino and Sandel 2017; Meregalli *et al.* 2020). The assignment of names to and descriptions of lobes has been based mainly on their position *vis-a-vis* the aedeagus – for example, the basal lobe, the median lobe, the lateral lobes, the apical lobe, and so on (Yamasako and Ohbayashi 2011; Daccordi *et al.* 2020; Meregalli *et al.* 2020). In the present study, the descriptions of the lobes and structures in their relation to the endophallus were made according to the nomenclature described by Cerezke (1964), whose criteria for naming the lobes were also based on their relational positions. The endophallus elements previously described for *Dendroctonus ponderosae* were the median lobe, the lateral lobes, the Y-structure, and the spines. In the descriptions made as part of the present study, the terms were adopted for the three genera that were analysed, and a general pattern of arrangement of two lateral lobes was recognised in *Dendroctonus, Ips,* and *Phloeosinus*. Although the scope of the nomenclature proposed in the present study could not be extrapolated to the entire subfamily, by the representation of a few taxa (Bollino and Sandel 2017; Meregalli *et al.* 2020), our data from three genera from different tribes (Hylurgini, Phloeosinini, and Ipini, *sensu* Hulcr *et al.* 2015) suggest that within Scolytinae, at least two lobes are maintained, and their numbers, positions, and shapes vary among genera and tribes. Therefore, some terms such as "median lobe" and "lateral lobes" apply to the whole group.

Another important point is the circular structure, or Y-structure, previously defined by Cerezke (1964) in the genus *Dendroctonus* and now described for the genus *Phloeosinus*. Although this structure is not the same in both genera, it is similar in shape and location and is probably a maintained feature within the subfamily Scolitynae.

Taxonomy

The endophallus is a key structure in the taxonomy and evolution of Coleoptera (Zhou *et al.* 2020; Bieńkowski 2021) and has been widely incorporated into species descriptions, phylogenetic reconstructions, and evolutionary biology studies because attributes present on the endophallus surface, such as sclerites, provide a rich source of taxonomic characteristics (Zunino and Halffter 1988; Coca-Abia 2007; Medina *et al.* 2013; Roggero *et al.* 2015; Uliana and Sabatinelli 2010).

The results of the present study support the use of endophallus morphology as a tool in taxonomy for the Scolytinae because each genus displays a particular pattern in the shape of the endophallus and because the arrangement, number, size, and shape of its lobes differ among genera and species. The inflation patterns display two different types of attachment between the aedeagus and the internal sac: in *Phloeosinus*, the base is attached to ventral folds around the ostium, and in *Dendroctonus* and *Ips*, the attachment of the sac membrane is by three or four distal lobes, respectively. In addition, the genera also differed in how the sacs retracted: in *Ips* specimens, the sac is retracted by the resistance applied by the ventral and dorsal lobes of the aedeagus, and in *Dendroctonus* and *Phloeosinus*, no mechanism of retraction is apparent, suggesting that retraction is due solely to the lack of seminal fluid pressure and muscular action (Cerezke 1964).

In *Dendroctonus*, the taxa with the most specimens examined in this study, the morphological patterns of the endophallus also suggest their value as phylogenetic characteristics. Endophallus similarities among species extend to those of the groups or complexes, as is supported with morphological, karyological, biological, and molecular characteristics as noted in Armendáriz-Toledano *et al.* (2014, 2015), Víctor and Zúñiga (2015), Godefroid *et al.* (2019), García-Román *et al.* (2019, 2022), Sullivan *et al.* (2021), and Ramírez-Reyes *et al.* (2023).

The species *D. vitei, D. mesoamericanus, D. frontalis, D. approximatus,* and *D. adjunctus* are grouped within the *Dendroctonus frontalis* complex (Lanier 1987; Víctor and Zúñiga 2016; Ramírez-Reyes *et al.* 2023). In the present study, this group was characterised by a rounded median lobe and two small lateral lobes arising in the dorsal–apical region of the median lobe. In *D. frontalis*, *D. mesoamericanus,* and *D. vitei*, members of *Dendroctonus frontalis* complex *sensu stricto* (Lanier 1987) or clades IV and V (Ramírez-Reyes *et al.* 2023), three similar lobes in position are present, but in *D. adjunctus*, *D. approximatus* and *D. barberi*, the other members of this complex corresponding to clade III (Ramírez-Reyes *et al.* 2023) share five lobes that are similar in position. However, in comparing the morphological patterns within these clades, discordances are observed in closely related species; for example, the endophallus of *D. vitei* is more similar to that of *D. mesoamericanus* than to *D. frontalis*, even though the latter two are sister species.

Members of the *D. valens* group also share endophallus similarities: *D. rhizophagus* and *D. valens* display three lobes, resembling a "T" and "Y" shape in dorsal view, a poorly developed median lobe, and two well-defined distal lateral lobes. Differences in lobe shape were observed among specimens of *D. valens* from Honduras and Mexico: the endophallus of *D. valens* from Honduras is more similar to those in *D. rhizophagus*: in both species, it is formed by three lobes resembling a "T" shape in ventral view, with the apical lateral lobe divided into two rounded small lobes equal in size and shape, whereas *D. valens* from Central Mexico displays an endophallus formed by three lobes resembling a "Y" shape in ventral view and two prominent distal lateral lobes with the apices ending in a point.

These morphological differences are consistent with the hypothesis that the Central American populations of *D. valens* correspond to a different species (Cai *et al.* 2008; Armendáriz-Toledano and Zúñiga 2017), which was previously recognised as *D. beckeri* by Thatcher (1954) and later synonymised with *D. valens* (Wood 1963).

Dendroctonus pseudotsugae barragani and *D. parallelocollis* present well-defined morphological patterns, distinct from those of *D. frontalis* and *D. valens* groups. The second species displays some shared attributes with the *D frontalis* complex: two small lateral rounded lobes attached to the distal end of the median lobe. In the most recently phylogeny, *D. parallelocollis* is the sister species of the *D. frontalis* complex (Ramírez-Reyes *et al.* 2023).

Sasabe *et al.* (2010) have proposed a possible lock-and-key role between the endophallus and bursa copulatrix in related species with variable endophallus morphology; however, Janovska *et al.* (2013) questions this theory for genera in which the endophallus is highly uniform. In the present study, we observed that the shape of the endophallus varies within the genus *Dendroctonus,* indicating that the lock-and-key condition may apply; however, examination of the shape and attributes that make up the bursa copulatrix is needed to corroborate whether the two structures complement each other.

In the genus *Phloeosinus,* the morphological differences among species are less evident and mostly are found in the shape of the endophallus lobes; however, more sister species pairs need to be studied to corroborate the lobes' taxonomic potential. Finally, the two *Ips* spp. examined in the present study display a less complex endophallus morphological pattern, with only two lateral lobes, which differ in the two species. Further analysis is required in other species of *Ips* to determine whether a pattern exists and to identify another, denser substance to inflate the endophallus that prevents its retraction and maintains its shape for a longer time to facilitate its anatomical study.

Although the present study elucidates the attributes of the endophallus and the importance of its study within the subfamily Scolytinae, further investigation into the possible evolutionary patterns of this structure is necessary to reveal how it may further the development of phylogenetic inferences.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.4039/tce.2024.27.

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References

Álvarez-Padilla, F. and Hormiga, G. 2007. A protocol for digesting internal soft tissues and mounting spiders for scanning electron microscopy. The Journal of Arachnology, **35**: 538–542.

Anichtchenko, A.V. 2010. Nueva especie de *Platyderus* Stephens, 1828 (Coleoptera Carabidae) de España y nuevos datos sobre *Platyderus toribioi* [New species of *Platyderus* Stephens, 1828 (Coleoptera Carabidae) from Spain and new data on *Platyderus toribioi*]. Arquivos Entomolóxicos, **3**: 103–106.

Armendáriz-Toledano, F., Niño, A., Macías Sámano, J.E., and Zúñiga, G. 2014. Review of the geographical distribution of *Dendroctonus vitei* (Curculionidae: Scolytinae) based on geometric morphometrics of the seminal rod. Annals of the Entomological Society of America, **107**: 748–755.

Armendáriz-Toledano, F., Niño, A., Sullivan, B.T., Kirkendall, L.R., and Zúñiga, G. 2015. A new species of bark beetle, *Dendroctonus mesoamericanus* sp. nov. (Curculionidae: Scolytinae), in southern Mexico and Central America. Annals of the Entomological Society of America, **108**: 403–414.

Armendariz-Toledano, F. and Zúñiga, G. 2017. Illustrated key to species of the genus *Dendroctonus* (Coleoptera: Curculionidae) occurring in Mexico and Central America. International Journal of Insect Science, **17**: 1–15. https://doi.org/10.1093/jisesa/iex009.

Berlov, O. 1992. Preparati permanenti a secco dell'endofallo nel genere *Carabus* L. (Coleoptera, Carabidae) [Permanent dry preparation of the endophallus in the genus *Carabus* L. (Coleoptera, Carabidae)]. Bolletino della Società Entomologica Italiana, **124**: 141–143.

Bieńkowski, A. 2021. The structure of the endophallus is a new promising feature and a key to study of taxonomy of the subgenus *Metallotimarcha* of the genus *Timarcha* (Coleoptera, Chrysomelidae) in the Caucasus. Insects, **12**: 937.

Blackman, M.W. 1942. Revision of the genus *Phloeosinus* Chapuis in North America (Coleoptera, Scolytidae). Proceedings of the United States National Museum, **92**: 397–474. https://doi.org/10.5479/si.00963801.92-3154.397.

Bollino, M. and Ruzzante, G. 2015. Corological notes on some species of *Pygopleurus* (Coleoptera: Glaphyridae) from the Greek Island of Lesbos. Munis Entomology and Zoology, **10**: 69–74.

Bollino, M. and Sandel, F. 2017. Two new taxa of the subgenus *Artapocyrtus* Heller, 1912, genus *Metapocyrtus* Heller, 1912 from the Philippines (Coleoptera, Curculionidae, Entiminae, Pachyrhynchini). Baltic Journal of Coleopterology, **17**: 1–14.

Bontems, C. 2013. Le procédé Berti–Vachon d'évagination du sac interne [The Berti–Vachon process of evagination of the internal sac]. Nouvelle Revue d'Entomologie (Nouvelle Série), **29**: 85–91.

Bukejs, A. and Anichtchenko, A. 2019. Description of fully inflated endophallus in some *Cassida* Linnaeus (Coleoptera: Chrysomelidae: Cassidinae). Baltic Journal of Coleopterology, **19**: 29–34.

Cai, Y.-W., Cheng, X.-Y., Xu, R.-M., Duan, D.-H., and Kirkendall, L.R. 2008. Genetic diversity and biogeography of red turpentine beetle *Dendroctonus valens* in its native and invasive regions. Insect Science, **15**: 291–301.

Cerezke, H.F. 1964. The morphology and functions of the reproductive systems *of Dendroctonus monticolae* Hopk. (Coleoptera: Scolytidae). The Canadian Entomologist, **96**: 477–500. https://doi.org/10.4039/Ent96477-3.

Cervantes-Espinoza, M., Ruiz, A.E., Cuellar-Rodríguez, G., Castro-Valderrama, U., and Armendáriz-Toledano, F. 2023. Immature stages of *Phloeosinus tacubayae* (Curculionidae: Scolytinae): morphology and chaetotaxy of larval pupae, sexual dimorphism of adults, and development time. Journal of Insect Science, **23**: 1–23.

Coca-Abia, M.M. 2007. Phylogenetic relationships of the subfamily Melolonthinae (Coleoptera, Scarabaeidae). Insect Systematics and Evolution, **38**: 447–472.

Coca-Abia, M.M. and Robbins, P.S. 2006. Taxonomy and phylogeny of a new Central American beetle genus: *Catrachia* (Coleoptera: Scarabaeidae). Revista de Biología Tropical, **54**: 519–529.

Cognato, A.I. and Smith, S.M. 2023. Taxonomic review of *Carphobius* Blackman, 1943 (Coleoptera: Curculionidae: Scolytinae) and a new species from San Clemente Island, California, USA. The Coleopterists Bulletin, **77**: 255–263.

Daccordi, M., Bollino, M., and Vela, J.M. 2020. Some techniques for the study of useful characters in the taxonomy of the genus *Timarcha* Samouelle, 1819 (Coleoptera, Chrysomelidae). European Journal of Taxonomy, **630**: 1–14. https://doi.org/10.5852/ejt.2020.630.

Danilevsky, M.L. and Kasatkin, D.G. 2004. Revision of the taxonomic structure of the tribe Dorcadionini (Coleoptera: Cerambycidae) on the base of endophallic morphology. Russian Entomological Journal, **13**: 127–149.

Douglas, H.B., Cognato, A.I., Grebennikov, V., and Savard, K. 2019. Dichotomous and matrix-based keys to the *Ips* bark beetles of the world (Coleoptera: Curculionidae: Scolytinae). Canadian Journal of Arthropod Identification, **38**: 1–234.

Düngelhoef, S. and Schmitt, M. 2010. Genital feelers: the putative role of parameres and aedeagal sensilla in Coleoptera Phytophaga (Insecta). Genetica, **138**: 45–57.

Erbey, M. and Candan, S. 2018. Comparative morphology of the endophallus (male internal genitalia) in eight species of the genus *Lixus* Fabricius, 1801 (Coleoptera: Curculionidae: Lixinae): a scanning electron microscope study. Life: The Excitement of Biology, **5**: 181–193.

Furniss, M.M. 1996. Taxonomic status of *Dendroctonus punctatus* and *D. micans* (Coleoptera: Scolytidae). Annals of the Entomological Society of America, **89**: 328–333.

Gao, C.B. and Coca-Abia, M.M. 2021. Revision of the genus *Miridiba* Reitter, 1902 (Coleoptera, Scarabaeidae, Melolonthinae): genital morphotypes and new taxonomic data. European Journal of Taxonomy, **749**: 1–94.

García-Román, J., Armendáriz-Toledano, F., Valerio-Mendoza, O., and Zúñiga, G. 2019. An assessment of old and new characters using traditional and geometric morphometrics for the identification of *Dendroctonus approximatus* and *Dendroctonus parallelocollis* (Curculionidae: Scolytinae). Journal of Insect Science, **19**: 14.

García-Román, J., Ramírez-Reyes, T., and Armendáriz-Toledano, F. 2022. The spermatheca in the genus *Dendroctonus* (Curculionidae: Scolytinae): morphology, nomenclature, potential characters for taxonomic use and phylogenetic signal. Revista Mexicana de Biodiversidad, **93**: 1–21.

Génier, F. 2019. Endophallites: a proposed neologism for naming the sclerotized elements of the insect endophallus (Arthropoda: Insecta). Annales de la Société entomologique de France, **55**: 482–484.

Godefroid, M., Meseguer, A.S., Sauné, L., Genson, G., Streito, J.C., Rossi, J.P., *et al.* 2019. Restriction-site associated DNA markers provide new insights into the evolutionary history of the bark beetle genus *Dendroctonus*. Molecular Phylogenetics and Evolution, **139**: 106528.

Hopkins, A.D. 1905. Notes on some Mexican Scolytidae: with descriptions of some new species. Proceedings of the Entomological Society of Washington, **7**: 71–81.

Hopkins, A.D. 1909. Contributions toward a monograph of the scolytid beetles: I. The genus *Dendroctonus*. Technical Series 17. Part I. United States Department of Agriculture, Bureau of Entomology, Washington, D.C., United States of America.

Hopping, G.R. 1963. Generic characters in the tribe Ipini (Coleoptera: Scolytidae), with a new species, a new combination, and new synonymy. The Canadian Entomologist, **95**: 61–68. https://doi.org/10.4039/Ent9561-1.

Hulcr, J., Atkinson, T.H., Cognato, A.I., Jordal, B.H., and McKenna, D.D. 2015. Morphology, taxonomy, and phylogenetics of bark beetles. *In* Bark Beetles. *Edited by* F.E. Vega and R.W. Hofstetter. Academic Press, Cambridge, Massachusetts, United States of America. Pp. 41–84.

Imura, Y. 2007. Endophallic structure of the genus *Platycerus* (Coleoptera, Lucanidae) of Japan, with descriptions of two new species. Elytra, **35**: 471–489.

Janovska, M., Anichtchenko, A.V., and Erwin, T. 2013. Significant new taxonomic tool for Carabidae (Insecta: Coleoptera): endophallus inflation methods revised. Caucasian Entomological Bulletin, **9**: 39–42.

Johnson, A.J., Hulcr, J., Knížek, M., Atkinson, T.H., Mandelshtam, M.Y., Smith, S.M., *et al.* 2020. Revision of the bark beetle genera within the former Cryphalini (Curculionidae: Scolytinae). Insect Systematics and Diversity, **4**: 1–81.

Johnson, P.L., Hayes, J.L., Rinehart, J., Sheppard, W.S., and Smith, S.E. 2008. Characterisation of two non-native invasive bark beetles, *Scolytus schevyrewi* and *Scolytus multistriatus* (Coleoptera: Curculionidae: Scolytinae). The Canadian Entomologist, **140**: 527–538. https://doi.org/10.4039/n07-034.

Jordal, B.H. 2024. Integrated taxonomy, biology, and biogeography of the Afrotropical genus *Xyloctonus* (Coleoptera, Curculionidae, Scolytinae). Deutsche Entomologische Zeitschrift, **71**: 67–84.

Jordal, B.H. 2021a. A phylogenetic and taxonomic assessment of Afrotropical Micracidini (Coleoptera, Scolytinae) reveals a strong diversifying role for Madagascar. Organisms Diversity & Evolution, **21**: 245–278.

Jordal, B.H. 2021b. Molecular and morphological revision of Afrotropical Hypoborini (Coleoptera: Curculionidae: Scolytinae) revealed novel bark beetle taxa with narrow geographical distributions. European Journal of Entomology, **118**: 90–110.

Justesen, M.J., Hansen, A.K., Knížek, M., Lindelow, Å., Solodovnikov, A., and Ravn, H.P. 2023. Taxonomic reappraisal of the European fauna of the bark beetle genus *Cryphalus* (Coleoptera, Curculionidae, Scolytinae). ZooKeys, **1179**: 63–105.

LaBonte, J.R. and Valley, S.A. 2019. Illustrated Key to the Species of *Ips*, *Orthotomicus*, and *Pseudips* of North America (or Spines, Spines, and More Spines). Oregon Department Agriculture, Salem, Oregon, United States of America. 80 pp.

Lanier, G.N. 1972. Biosystematics of the genus *Ips* (Coleoptera: Scolytidae) in North America. Hopping's groups IV and X. The Canadian Entomologist, **104**: 361–388. https://doi.org/10.4039/Ent104361-3.

Lanier, G.N. 1987. The validity of *Ips cribricollis* (Eich.)(Coleoptera: Scolytidae) as distinct from *I. grandicollis* (Eich.) and the occurrence of both species in Central America. The Canadian Entomologist, **119**: 179–187. https://doi.org/10.4039/Ent119179-2.

Lanier, G.N., Teale, S.A., and Pajares, J.A. 1991. Biosystematics of the genus *Ips* (Coleoptera: Scolytidae) in North America: review of the *Ips calligraphus* group. The Canadian Entomologist, **123**: 1103–1124. https://doi.org/10.4039/Ent1231103-5.

Lyon, R.L. 1958. A useful secondary sex character in *Dendroctonus* bark beetles. The Canadian Entomologist, **90**: 582–584. https://doi.org/10.4039/Ent90582-10.

Mandelshtam, M.Y. and Petrov, A.V. 2019. A key to species of the tribe Hylastini LeConte, 1876 (Coleoptera: Curculionidae: Scolytinae) from Russia and adjacent countries. Russian Entomological Journal, **28**: 389–399.

Mandelshtam, M.Y., Petrov, A.V., Axentjev, S.I., and Knížek, M. 2006. A new and a poorly known species of bark beetles (Coleoptera: Scolytidae) from Middle Asia. Proceedings of the Russian Entomological Society, **77**: 213–218.

Mandelshtam, M.Y., Petrov, A.V., and Korotyaev, B.A. 2012. To the knowledge of the herbivorous scolytid genus *Thamnurgus* Eichhoff (Coleoptera, Scolytidae). Entomological Review, **92**: 329–349.

Medina, C.A., Molano, F., and Scholtz, C.H. 2013. Morphology and terminology of dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) male genitalia. Zootaxa, **3626**: 455–476.

Meregalli, M., Boriani, M., Taddei, A., Hsu, C.F., Tseng, W.Z., and Mouttet, R. 2020. A new species of *Aclees* from Taiwan with notes on other species of the genus (Coleoptera: Curculionidae: Molytinae). Zootaxa, **4868**: 1–26. https://doi.org/10.11646/ZOOTAXA.4868.1.1.

Nichols, S.W. 1989. The Torre-Bueno Glossary of Entomology. Revised edition. New York Entomological Society, New York, New York, United States of America. 840 pp.

Pajares, J.A. and Lanier, G.N. 1990. Biosystematics of the turpentine beetles *Dendroctonus terebrans* and *D. valens* (Coleoptera: Scolytidae). Annals of the Entomological Society of America, **83**: 171–188.

Pardo-Diaz, C., Toro, A.L., Tovar, S.A.P., Sarmiento-Garcés, R., Herrera, M.S., and Salazar, C. 2019. Taxonomic reassessment of the genus *Dichotomius* (Coleoptera: Scarabaeinae) through integrative taxonomy. PeerJ, **7**: e7332.

Pérez-Silva, M., Equihua Martínez, A., and Atkinson, T.H. 2015. Identificación de las especies mexicanas del género *Xyleborus* Eichhoff, 1864 (Coleoptera: Curculionidae: Scolytinae) [Identification of the Mexican species of the genus *Xyleborus* Eichhoff, 1864 (Coleoptera: Curculionidae: Scolytinae)]. Insecta Mundi, **440**: 1–35.

Ramírez-Reyes, T., Armendáriz-Toledano, F., and Rodríguez, L.G.C. 2023. Rearranging and completing the puzzle: phylogenomic analysis of bark beetles *Dendroctonus* reveals new hypotheses about genus diversification. Molecular Phylogenetics and Evolution, **187**: 107885.

Rios-Reyes, A.V., Valdez-Carrasco, J., Equihua-Martínez, A., and Moya-Raygoza, G. 2008. Identification of *Dendroctonus frontalis* (Zimmermann) and *D. mexicanus* (Hopkins) (Coleoptera: Curculionidae: Scolytinae) through structures of the female genitalia. The Coleopterists Bulletin, **62**: 99–103.

Roggero, A., Barbero, E., and Palestrini, C. 2015 Phylogenetic and biogeographical review of the Drepanocerina (Coleoptera: Scarabaeidae: Oniticellini). Arthropod Systematics and Phylogeny, **73**: 153–174.

Sasabe, M., Takami, Y., and Sota, T. 2010. QTL for the species-specific male and female genital morphologies in *Ohomopterus* ground beetles. Molecular Ecology, **19**: 5231–5239.

Sharp, D. and Muir, F. 1912. XI. The comparative anatomy of the male genital tube in Coleoptera. Transactions of the Royal Entomological Society of London, **60**: 477–642.

Smith, S.M. and Cognato, A.I. 2010. A taxonomic revision of *Camptocerus* Dejean (Coleoptera: Curculionidae: Scolytinae). *Insecta Mundi*, **0148**: 1–88.

Smith, S.M. and Cognato, A.I. 2014. A taxonomic monograph of Nearctic *Scolytus* Geoffroy (Coleoptera, Curculionidae, Scolytinae). ZooKeys, **450**: 1–182.

Sullivan, B.T., Grady, A.M., Hofstetter, R.W., Pureswaran, D.S., Brownie, C., Cluck, D., and Zúñiga, G. 2021. Evidence for semiochemical divergence between sibling bark beetle species: *Dendroctonus brevicomis* and *Dendroctonus barberi*. Journal of Chemical Ecology, **47**: 10–27.

Thatcher, T.O. 1954. A new species of *Dendroctonus* from Guatemala (Scolytidae). Publication No. 6. The Coleopterists Bulletin, **8**: 3–6.

Tuxen, S.L. 1970. Taxonomist's Glossary of Genitalia in Insects. Lubrecht & Cramer Limited, Port Jervis, New York, United States of America.

Uliana, M. and Sabatinelli, G. 2010. Revision of *Eulasia genei* Truqui, with description of *Eulasia rittneri* **n. sp.** from Israel and synonymic notes on related species (Coleoptera: Scarabaeoidea: Glaphyridae). Zootaxa, **2436**: 28–56.

Valerio-Mendoza, O., García-Román, J., Becerril, M., Armendáriz-Toledano, F., Cuéllar-Rodríguez, G., Negrón, J. F., *et al.* 2019. Cryptic species discrimination in western pine beetle, *Dendroctonus brevicomis* LeConte (Curculionidae: Scolytinae), based on morphological characters and geometric morphometrics. Insects, **10**: 377.

Van Dam, M.H. 2014. A simple, rapid technique for the inflation of the endophallus, with particular focus on the Curculionoidea (Coleoptera). The Coleopterists Bulletin, **68**: 263–268.

Víctor, J. and Zúñiga, G. 2016. Phylogeny of *Dendroctonus* bark beetles (Coleoptera: Curculionidae: Scolytinae) inferred from morphological and molecular data. Systematic Entomology, **41**: 162–177.

Wood, S.L. 1957. Ambrosia beetles of the tribe Xyloterini (Coleoptera: Scolytidae) in North America. The Canadian Entomologist, **89**: 337–354. https://doi.org/10.4039/Ent89337-8.

Wood, S.L. 1963. A revision of the bark beetle genus *Dendroctonus* Erichson (Coleoptera: Scolytidae). The Great Basin Naturalist, **23**: 1–117.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae): a taxonomic monograph. The Great Basin Naturalist Memoirs, **6**: 1–1359.

Yamasako, J. and Ohbayashi, N. 2011. Review of the genus *Paragolsinda* Breuning, 1956 (Coleoptera, Cerambycidae, Lamiinae, Mesosini), with reconsideration of the endophallic terminology. Zootaxa, **2882**: 35–50.

Yang, G., Yang, X., and Shi, H. 2020. Taxonomy and phylogeny of the genus *Gastrocentrum* Gorham (Coleoptera, Cleridae, Tillinae), with the description of five new species. ZooKeys, **979**: 99–132.

Zhou, Y.L., Zhou, H.Z., Ski, A.L., and Beutel, R.G. 2020. Evolution of a hyper-complex intromittent organ in rove beetles: the endophallus of Xantholinini (Staphylinidae: Coleoptera). Zoological Journal of the Linnean Society, **188**: 1277–1295.

Zunino, M. and Halffter, G. 1988. Análisis taxonómico, ecológico y biogeográfico de un grupo americano de *Onthophagus* (Coleoptera: Scarabaeidae) [Taxonomic, ecological and biogeographical analysis of an American group of *Onthophagus* (Coleoptera: Scarabaeidae)]. Volume 9. Monografie di Museo Regionale di Scienze Naturali. Museo Regionale di Scienze Naturali, Torino, Italy. 213 pp.

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